

# Synthesis and reactions of uronic acid derivatives. XVII.\*

## Synthesis of methyl 2-*O*-(methyl 4-*O*-methyl- $\alpha$ - and $\beta$ -D-glucopyranosyluronate)- $\beta$ -D-xylopyranoside

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Acetolysis of methyl (benzyl 2,3-di-*O*-benzyl-4-*O*-methyl- $\beta$ -D-glucopyranosid)uronate (*IV*) gave the corresponding anomeric acetates *VI* and *VII*. The same acetates were obtained in a better overall yield from benzyl 2,3-di-*O*-benzyl-4-*O*-methyl- $\beta$ -D-glucopyranosiduronic acid (*III*) by acid hydrolysis, treatment of the product with ethereal diazomethane, and acetylation of the resulting methyl ester *V*. Treatment of *VI* and *VII* with hydrogen bromide in dichloromethane or of *V* with thionyl chloride afforded glycosyl halides *VIII* and *XV*, respectively. Compounds *VIII* and *XV* derived from 4-*O*-methyl-D-glucuronic acid, bearing a benzyloxy group at C-2, nonparticipating in the modified Koenigs—Knorr synthesis of glycosides, were tested in the chemical synthesis of a 4-*O*-methyl- $\alpha$ -D-glucuronopyranosyl linkage. The reaction of bromide *VIII* with methyl 3,4-di-*O*-acetyl- $\beta$ -D-xylopyranoside in nitromethane—benzene in the presence of mercuric cyanide gave the  $\alpha$ - and  $\beta$ -linked oligosaccharides *IX* and *X* in a ratio of 1.4:1. Much better stereoselectivity ( $\alpha$ : $\beta$  = 18:1) was observed in the reaction of chlorides *XV* with the same nucleophile when the reaction was carried out in dry ether in the presence of silver perchlorate. Catalytic hydrogenolysis of *IX* and *X* followed by deacetylation gave the title, crystalline aldobiouronic acid derivatives *XII* and *XIV* which were further characterized as the acetates *XI* and *XIII*.

Результатом ацетоллиза метил(бензил 2,3-ди-*O*-бензил-4-*O*-метил- $\beta$ -D-глюкопиранозид)уроната (*IV*) являются соответствующие аномерные ацетаты *VI* и *VII*. Те же самые ацетаты были получены в существенно высших выходах из бензил 2,3-ди-*O*-бензил-4-*O*-метил- $\beta$ -D-глюкопиранозидуроновой кислоты (*III*) способом кислого гидролиза, действием эфирного раствора диазометана и последующим ацетилированием возникшего метилэфира *V*. Реакцией веществ *VI* и *VII* с бромистым водородом в дихлорметане или вещества *V* с тионилхлоридом получают гликозилгалогениды *VIII* или *XV*. Была проверена удобность в химическом синтезе 4-*O*-метил- $\alpha$ -D-глюкопиранозидовой связи, производных 4-*O*-метил-D-глюкуроновой кислоты *VIII* и *XV*, носящих оксисбензиль-

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ную группу на C-2, не принимающую участие в синтезе гликозидов по Кенигсу–Кнорру. Реакцией бромида VIII с метил-3,4-ди-*O*-ацетил- $\beta$ -D-ксилопиранозидом в нитрометан–бензоле в присутствии цианида ртути получают  $\alpha$ - и  $\beta$ -связаны олигосахариды IX и X в отношении 1,4:1. Лучшая стереоселективность ( $\alpha$ : $\beta$  = 18:1) была замечена при реакции хлоридов XV с тем же самым нуклеофилом в случае реакции в сухом эфире в присутствии перхлората серебра. Каталитическим гидрогенолизом веществ IX и X с последующим деацетилированием получились кристаллические производные XII и XIV альдобииуроновой кислоты, которые были охарактеризованы в качестве ацетатов XI и XIII.

The basic structural unit of (4-*O*-methylglucurono)xylans, the most abundant hemicellulose species of hardwoods, is 2-*O*-(4-*O*-methyl- $\alpha$ -D-glucuronopyranosyl)-D-xylopyranose which is  $\beta$ -glycosidically linked in the polysaccharide chain [2]. Oligosaccharides related to the afore-mentioned aldobiouronic acid were obtained from the products of partial hydrolysis of wood xylans [3–6] but their synthesis by chemical methods has not been described. Here we report on the first chemical synthesis of aldobiouronic acid derivatives with  $\alpha$ -linked 4-*O*-methyl-D-glucuronic acid. The title compound XII (Scheme 2) was needed as a model in studies related to chemical processing of woods containing (4-*O*-methylglucurono)xylans.

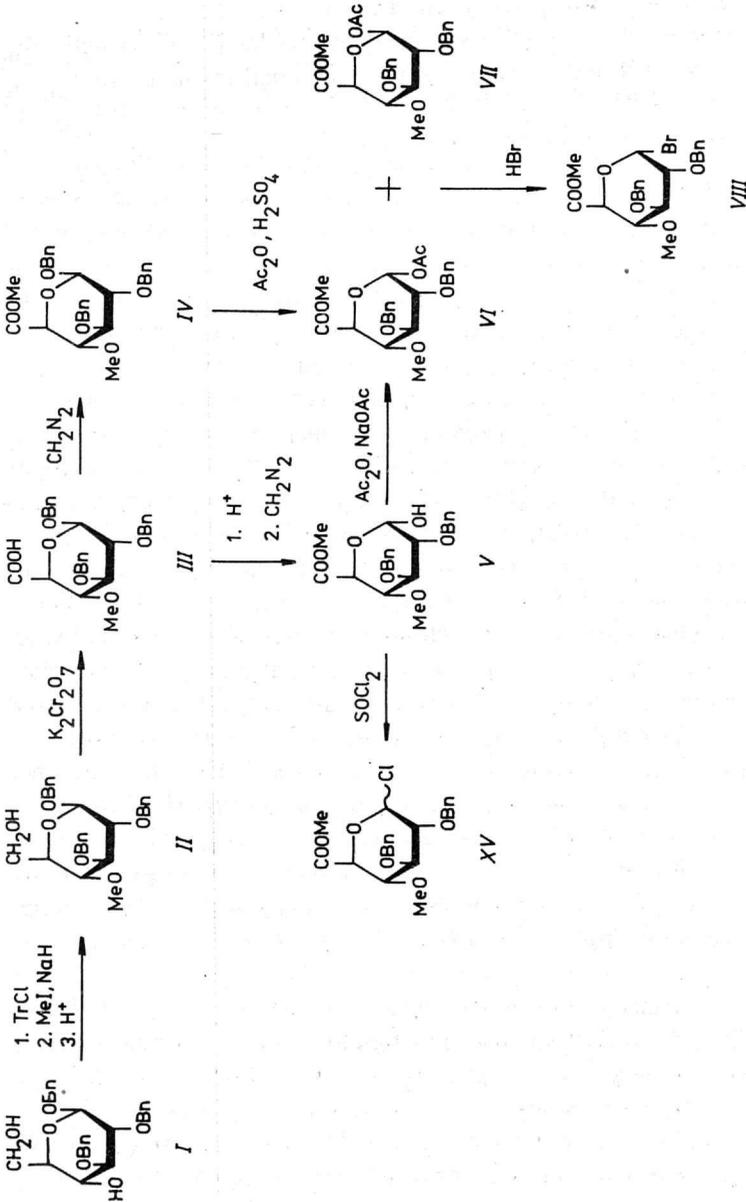
Synthesis of the aldobiouronic acid derivative with 4-*O*-methyl- $\alpha$ -D-glucuronic acid linked to D-xylose at C-2 required two types of starting substances: a D-xylose derivative bearing easily removable blocking groups and having only HO-2 unsubstituted (syntheses of compounds of this class have been reported from this laboratory [7, 8]), and a suitable glycosyl halide derived from 4-*O*-methyl-D-glucuronic acid. A glycosyl halide ( $\alpha$ -bromide per *O*-acetate) derived from 4-*O*-methyl-D-glucuronic acid was first prepared by *Bochkov* and *Voznyi* [9]. Syntheses of other, crystalline substances of this class suitable, however, owing to the presence of an acyloxy group at C-2 preferentially for the preparation of  $\beta$ -linked oligosaccharides were described elsewhere in this Series [10]. Of the available methods of chemical synthesis of  $\alpha$ -linked oligosaccharides [11] modifications of Koenigs–Knorr synthesis of glycosides involving glycosyl halides bearing a nonparticipating group at C-2 appear to be preparatively most feasible. To our knowledge such a halide derived from 4-*O*-methyl-D-glucuronic acid has not been described in the literature. Although the synthesis of a substance of this class from D-glucose is rather complicated, this approach is by all means more practicable than the isolation of 4-*O*-methyl-D-glucuronic acid from a natural source and its further conversion. It requires that the functional groups (carboxyl or its ester at C-5, methoxyl at C-4, and a nonparticipating group at C-2) be located at the proper positions before a halogen is introduced at C-1. Moreover, a functional group

capable of an exchange for a halogen must necessarily be at the anomeric position. Since by means of splitting glycosides with dihalogenomethyl methyl ethers [12, 13] a glycosyl halide could not be obtained from the known [14] methyl (benzyl 2,3-di-*O*-benzyl-4-*O*-methyl- $\beta$ -D-glucopyranosid)uronate (*IV*) the next approach to the synthesis of a substance fulfilling the above-mentioned requirements, namely of methyl 1-*O*-acetyl-2,3-di-*O*-benzyl-4-*O*-methyl- $\alpha$ - or - $\beta$ -D-glucopyranuronate, was the acetolysis of *IV*. We have conducted the reaction catalyzed by mineral [15] or Lewis acids [16] under various conditions, taking into account the results of others [17, 18] according to which the outcome of acetolysis depends greatly not only upon the nature of the catalyst, reaction time, and temperature but also on the amount of acetic anhydride and, if used, inert solvent. The formation of complex reaction mixtures was observed by monitoring the reactions by t.l.c. and the p.m.r. spectra of products isolated by chromatography showed that these were, most of the time, mixed acetates containing more than one acetyl group per a molecule. Some of them were related to acylals isolated occasionally [17—19] from similar reactions. Although the wanted  $\alpha$ - and  $\beta$ -acetates *VI* and *VII* could be eventually obtained in this way from *IV* the yields of pure substances were not satisfactory and the procedure requiring the isolation of *VI* and *VII* by chromatography is impractical in a large-scale preparation. Good yields of *VI* and *VII* were obtained by a new reaction pathway (Scheme 1) starting with the known [20] benzyl 2,3-di-*O*-benzyl- $\beta$ -D-glucopyranoside (*I*). Through a series of conventional reactions compound *I* was converted to its 4-*O*-methyl derivative *II* which, when oxidized with potassium bichromate, afforded crystalline easily isolable benzyl 2,3-di-*O*-benzyl-4-*O*-methyl- $\beta$ -D-glucopyranosiduronic acid (*III*). Treatment of *III* with diazomethane gave the methyl ester *IV* identical with the substance prepared in an independent manner [14]. The benzyl glycoside *III* was hydrolyzed with an acetic acid—trifluoroacetic acid—water mixture and, without isolation, the product was treated with ethereal diazomethane to give the hitherto unknown, crystalline ester *V*. When *V* was acetylated in a conventional manner the acetates *VI* and *VII*, identical with those isolated from the products of acetolysis of *IV*, were produced.

Treatment of the crystalline acetate *VII* or of a mixture of *VI* and *VII* with hydrogen bromide in dichloromethane afforded then the glycosyl bromide *VIII*.

A stereoselective synthesis of  $\alpha$ -D-glucopyranosides from  $\alpha$ - or  $\beta$ -D-glucopyranosyl bromides bearing a nonparticipating group at C-2 was described by *Flowers* [21]. Reaction of *VIII* with methyl 3,4-di-*O*-acetyl- $\beta$ -D-xylopyranoside [8] (Scheme 2) under these conditions was a smooth reaction and gave a satisfactory yield of the wanted oligosaccharides but with low stereoselectivity. The formed aldobiouronic acid derivatives *IX* and *X*, isolated by chromatography of the reaction mixture in 69% yield, were obtained in a ratio of 1.4:1.

Recent works of *Igarashi et al.* [22, 23] indicated that anomeric glycosyl



Me — methyl  
Tr — trityl

Ac — acetyl  
Bn — benzyl

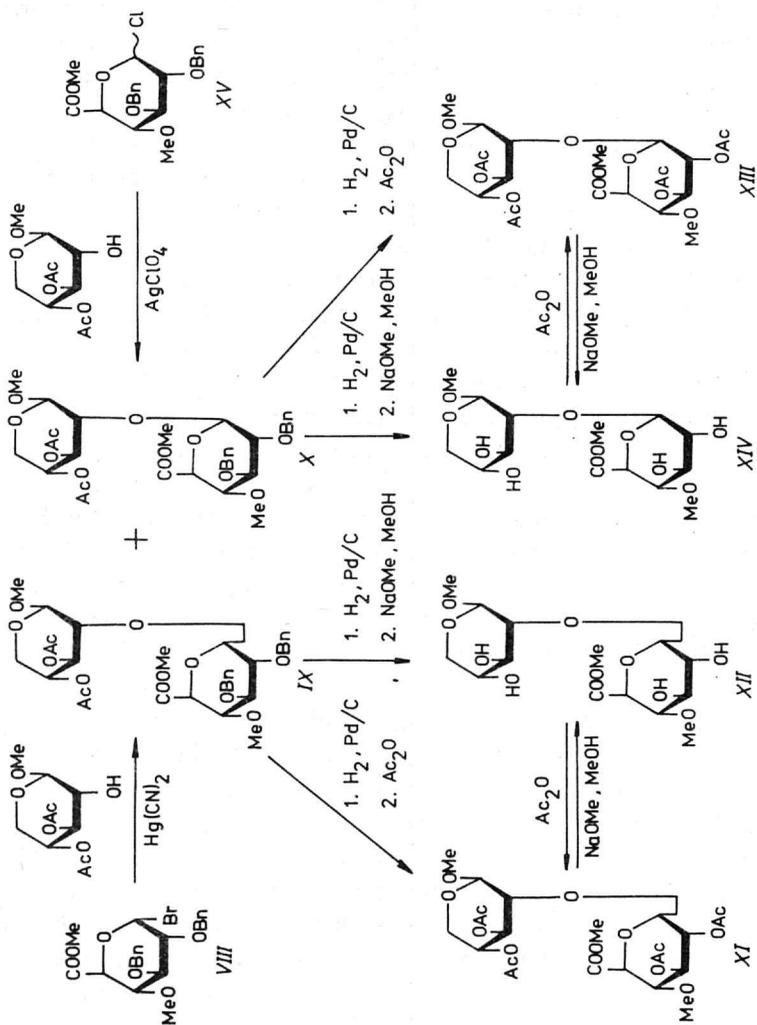
Scheme 1

chlorides or perchlorates might be generally useful in the preparation of  $\alpha$ -glucopyranosides. The reaction [22] of glycosyl chlorides with nucleophiles in ether in the presence of silver perchlorate gave simple  $\alpha$ -glycosides ( $\sim 90\%$ ) with high stereoselectivity and  $\alpha$ -glucooligosaccharides were obtained in the same manner [23]. Condensation of the chlorides *XV*, obtained by treatment of *V* with thionyl chloride, with methyl 3,4-di-*O*-acetyl- $\beta$ -D-xylopyranoside (Scheme 2) confirmed the results of the original authors. The reaction was fast and highly stereoselective: crystalline oligosaccharides *IX* and *X* were obtained in the ratio of 18:1 and combined yield  $\sim 90\%$ . Considering, in addition, the experimental simplicity of the reaction, for making  $\alpha$ -glucopyranosides, the procedure is unquestionably superior to the conversions developed earlier [11, 21, 24].

Catalytic hydrogenolysis of *IX* and subsequent deacetylation (Zemplén) afforded the wanted glycoside *XII*. The aldobiouronic acid derivative *XIV* was prepared following the same reaction pathway. Compounds *XII* and *XIV* were further characterized as crystalline acetates *XI* and *XIII* the mass spectra of which were qualitatively identical and consistent with their structure [25].

The proof of the configuration at the interglycosidic linkage in *IX*—*XIV* is based on p.m.r. data, namely on the magnitude of the coupling constants  $J_{1,2}$  and  $J_{1',2'}$  found in the spectra of the deblocked glycosides *XII* and *XIV*. The assignment of the doublets appearing at  $\delta$  4.42 and 4.41 ( $J$  7.3 and 7.2 Hz, respectively) to H-1 of the substances was based on the similarity with the chemical shift of H-1 ( $\delta$  4.28,  $J_{1,2}$  6.7 Hz) found in the spectrum of methyl 3,4-di-*O*-acetyl- $\beta$ -D-xylopyranoside [8]. The signals at  $\delta$  5.25 and 4.71 in the spectra of *XII* and *XIV* were assigned to H-1' by analogy with similar D-glucuronic acid derivatives [13, 19]. From the coupling constants  $J_{1',2'}$  found in the spectra (3.2 and 7.4 Hz) follows the  $\alpha$ - and  $\beta$ -interglycosidic linkage for *XII* and *XIV*, respectively.

By treatment with methanolic hydrogen chloride of 2-*O*-(4-*O*-methyl- $\alpha$ -D-glucuronopyranosyl)-D-xylopyranose isolated from wood (4-*O*-methylglucuronoxylan Timell [26] obtained a sirupy methyl ester methyl glycoside ( $[\alpha]_D + 77^\circ$ ). Following acetylation gave then a crystalline acetate (m.p. 200°C,  $[\alpha]_D + 100^\circ$ ) which, when prepared to characterize the amorphous aldobiouronic acid isolated from the products of partial hydrolysis of wood xylans [e.g. 26—28] is always referred to without specifying the anomeric configuration at the D-xylose moiety. Elsewhere [2] for the same acetate a formula is given corresponding to methyl 2-*O*-(methyl 2,3-di-*O*-acetyl-4-*O*-methyl- $\alpha$ -D-glucopyranosyluronate)-3,4-di-*O*-acetyl- $\alpha$ -D-xylopyranoside. The physical constants found for *XI* and *XII*, together with the i.r. spectrum of *XI* which was superimposable with that of the substance obtained by the conversion of the material isolated from a natural source (kindly supplied by Professor Timell) suggests, that Timell's acetate [26] is identical with the methyl  $\beta$ -D-xylopyranoside derivative described herein.



Scheme 2

## Experimental

Melting points were determined on a Kofler hot-stage. Optical rotations were measured with a Perkin—Elmer automatic polarimeter, Model 141. P.m.r. spectra (Table 1) for solutions in chloroform-*d* or D<sub>2</sub>O [internal standard tetramethylsilane and sodium 3-(trimethylsilyl)propionate-*d*<sub>4</sub>, respectively] were measured at 80 MHz using a Tesla BS 487-B spectrometer. Proton-signal assignments were made by the INDOR technique. Mass spectra (70 eV) were obtained with a JMS 100 D mass spectrometer. I.r. spectra for samples in KBr pellets were recorded using a Perkin—Elmer spectrometer, Model 457.

Thin-layer chromatography (t.l.c.) was performed on Silica gel G, and column chromatography on dry-packed Kieselgel 60 (Merck, A. G., Darmstadt, Cat. No. 9385), with: *A*. benzene—acetone 15:1, *B*. heptane—methyl acetate 4:1, *C*. heptane—benzene 1:2, *D*. benzene—methanol 4:1, *E*. heptane—acetone 4:1, *F*. benzene—methyl acetate 30:1, *G*. benzene—acetone 25:1, *H*. chloroform—acetone 5:1, and *I*. chloroform—methanol 6:1. Detection was done by spraying with 5% (v/v) sulfuric acid in ethanol and heating until permanent char spots were visible. Prior to packing, Kieselgel 60 was equilibrated with 40% (v/w) of the mobile phase, instead of the recommended 10% [29]. The amount of the adsorbent used in preparative chromatography was 100-fold, based on the amount of the chromatographed mixture. Solutions were concentrated at 40°C/2 kPa. Ether used in the reaction of *XV* with methyl 3,4-di-*O*-acetyl-β-*D*-xylopyranoside was refluxed over powdered sodium hydride and distilled. Silver perchlorate was freshly prepared by the procedure which is a timesaving modification of the known procedure [30]: a suspension of silver carbonate [31] (5.5 g) in water (50 ml) was stirred in the dark at 90—95°C and perchloric acid (70%, 3.9 ml) was added dropwise. The mixture was heated for 1 h and then filtered through a fine-porosity sintered-glass funnel. The solution was concentrated with the exclusion of direct light, and dehydrated by co-evaporation with toluene. A small amount of material insoluble in benzene was filtered and pentane was added to the filtrate. The white precipitate was collected by filtration and dried at 80—90°C/2 kPa.

### *Benzyl 2,3-di-O-benzyl-4-O-methyl-β-D-glucopyranoside (II)*

Trityl chloride (70 g; 0.25 mole) was added with stirring to the solution of *I* (100 g; 0.22 mole) in dry pyridine (300 ml) and the solution was heated with the exclusion of moisture at 90°C until t.l.c. (solvent *A*) showed almost complete conversion of the starting material (*R<sub>f</sub>* 0.1) to the product (*R<sub>f</sub>* 0.8) (~4 h). The mixture was worked up in the usual manner and sodium hydride (30 g), followed by methyl iodide (30 ml) was added to the solution of the crude product in 1,2-dimethoxyethane (1500 ml). The mixture was stirred under gentle reflux with the exclusion of atmospheric moisture and carbon dioxide until t.l.c. (solvent *B*) showed that the starting sugar component (*R<sub>f</sub>* 0.3) has all reacted to the product (*R<sub>f</sub>* 0.4) (~4 h). The excess of the methylation reagent was destroyed by careful addition of methanol and the organic solvents were removed from the formed clear solution. The water phase was neutralized with acetic acid (pH 8), extracted with chloroform, the chloroform solution was backwashed with water (3 × 300 ml) and concentrated. Water was added at 90—100°C to slight turbidity to the solution of the residue in acetic acid (750 ml) and the

Table 1

Chemical shifts and first-order coupling constants for II—VIII (CDCl<sub>3</sub>)<sup>a</sup>

Compound	Chemical shifts <sup>b</sup> ( $\delta$ )											Coupling constants (Hz)						
	H-1	H-2	H-3	H-4	H-5	H-6,6'	OCH <sub>3</sub>	OAc	OH	Arom.	CH <sub>2</sub>	$J_{1,2}$	$\bar{J}_{2,3}$	$J_{3,4}$	$J_{4,5}$			
II	4.53 bd <sup>c</sup>	3.20	—————			3.94 m	3.53	—	2.55	7.25 <sup>d</sup>	4.91 d; 4.64 d;	4.91 d; 4.70 d;	4.87 d; 4.81 d	~7.0	e	e	e	
III	4.58 bd <sup>c</sup>	3.45	—————			4.05 m	—	3.54	—	10.05	7.26 <sup>d</sup>	4.96 d; 4.64 d;	4.89 d; 4.70 d;	4.85 d; 4.78 d	~7.0	e	e	e
IV	4.54 d	3.51 q	3.90 t	3.44 t	4.38 d	—	3.45; 3.71	—	4.25	7.30 <sup>f</sup>	4.84 d;	4.64 s <sup>g</sup> ;	4.78 d	~7.0	9.5	8.5	9.7	
V	4.53 bd <sup>c</sup>	3.40	—————			4.00 m	—	3.48; 3.78	—	—	7.30 <sup>d</sup>	4.91 d; 4.60 d;	4.88 d; 4.67 d;	4.84 bd; 4.76 d	~7.0	e	e	e
VI	6.33 d	3.68 q	3.91 t	3.51 t	4.27 d	—	3.53; 3.81	2.13	—	7.30 <sup>f</sup>	4.96 d;	4.71 s <sup>g</sup> ;	4.88 d	3.1	9.3	8.6	9.8	
VII	5.69 bd <sup>c</sup>	3.45	—————			4.20 m	—	3.53; 3.83	2.04	—	7.30 <sup>f</sup>	4.93 s <sup>g</sup> ;	4.83 s <sup>g</sup>		~7.0	e	e	e
VIII	6.31 d	3.48 q	3.80 t	3.51 t	4.36 d	—	3.48; 3.74	—	—	7.30 <sup>f</sup>	4.87 d;	4.63 s <sup>g</sup> ;	4.76 d	3.1	9.5	8.5	9.9	

a) Data obtained from spectra recorded at a sweep width 100 Hz.

b) Observed multiplicities: s — singlet, d — doublet, t — triplet, q — quartet, m — multiplet, b — broadened.

c) Broadening owing to a second-order effect.

d) 15-Proton multiplet.

e) First-order couplings unobserved.

f) 10-Proton multiplet.

g) 2-Proton singlet.

solution was kept at the same temperature for 4 h after which time t.l.c. (solvent *B*) showed that the detritylation was complete. The reaction mixture was cooled to room temperature, the separated triphenyl methanol was filtered, washed with ethanol and the filtrate was concentrated with co-evaporation with water to remove acetic acid. The 6-*O*-acetate, formed spontaneously on concentration of the solution containing dilute acetic acid ( $R_f$  0.4, cf. 0.2 for the product *II* of detritylation, solvent *A*), was deacetylated (Zemplén) and *II*, after decoloration with a little silica gel, was crystallized from benzene—heptane. The obtained material (39 g) was sufficiently pure for the next step. Chromatography of the product in the mother liquor (subsequent elution with solvent *C*, benzene, and solvent *A*) gave a second crop of *II* (17 g, total yield 54%, based on *I*). A portion, when recrystallized from methanol, melted at 112–113°C and had  $[\alpha]_D^{22} + 10^\circ$  ( $c$  1, chloroform).

For  $C_{28}H_{32}O_6$  (464.54) calculated: 72.39% C, 6.94% H; found: 72.12% C, 6.93% H.

*Benzyl 2,3-di-O-benzyl-4-O-methyl-β-D-glucopyranosiduronic acid (III)  
and its methyl ester (IV)*

A solution of potassium bichromate (4.25 g) in 3.5 M sulfuric acid (16.5 ml) was added with stirring to the solution of *II* (5 g) in acetone (80 ml). The solution was stirred for 5 min and then heated at 55°C for 1 h. After cooling to room temperature, when the solid settled, the supernatant solution was slowly poured into vigorously stirred water (300 ml), the chromium salts were washed with acetone ( $2 \times 5$  ml) and the washings were combined with the product. The white crystalline solid was filtered off, washed with water (twice), dissolved in chloroform, the chloroform solution was washed with water (twice), dried, and concentrated. T.l.c. (solvent *D*) of the crystalline residue (5 g) showed the presence of only trace amounts of substances other than *III* (tailing spot at  $R_f \sim 0.6$ ) which was sufficiently pure for the next step. Crystallization from chloroform—heptane gave 4.7 g (91%) of pure *III*, m.p. 148–149°C (after recrystallization from isopropyl ether),  $[\alpha]_D^{22} - 20^\circ$  ( $c$  1, chloroform).

For  $C_{28}H_{30}O_7$  (478.50) calculated: 70.27% C, 6.32% H; found: 70.40% C, 6.46% H.

The reaction was carried out without complications with 30 g of *II* and *III* was obtained in the same yield.

A portion of the acid *III* (0.3 g) in methanol (5 ml) was treated with ethereal diazomethane. After concentration and recrystallization of the solid residue from chloroform—methanol *IV* melted at 112–113°C. Ref. [14] m.p. 113–113.5°C.

*Methyl 2,3-di-O-benzyl-4-O-methyl-α-D-glucopyranuronate (V)*

Water (20 ml) was added at 100°C to the solution of the crude product of oxidation of *II* (5 g) in acetic acid—trifluoroacetic acid (15:1; 80 ml) and the solution was kept at 100–105°C for 24 h. T.l.c. (solvent *D*) showed then that one major product ( $R_f \sim 0.4$ , tailing) was formed. The reaction mixture was diluted with water (100 ml), concentrated with co-distillation with water to remove volatile organic acids and benzyl alcohol, and ethereal diazomethane was added at 0°C to the solution of the residue in methanol (50 ml).

When the evolution of nitrogen ceased, indicating complete methyl ester formation, the solution was quickly concentrated and the solution of the residue in benzene, containing one major product ( $R_f$  0.3; solvent A), was treated with a little silica gel. Crystallization from isopropyl ether gave 2.1 g of white V. Chromatography of the product in the mother liquor gave more V (1 g, total yield ~70%, based on II). After recrystallization from the same solvent V melted at 107.5–108.5°C and had  $[\alpha]_D^{25} + 26^\circ$  (c 1, chloroform).

For  $C_{22}H_{26}O_7$  (402.43) calculated: 65.66% C, 6.51% H; found: 65.74% C, 6.51% H.

*Methyl 1-O-acetyl-2,3-di-O-benzyl-4-O-methyl- $\alpha$ - (VI) and - $\beta$ -D-glucopyranuronate (VII)*

a) A mixture of sulfuric acid and glacial acetic acid (1:10; 0.5 ml) was added to the solution of benzyl glycoside IV (1 g) in dry, alcohol-free chloroform (5 ml) containing glacial acetic acid (2 ml) and acetic anhydride (0.6 ml), and the reaction mixture was kept at 20°C for 48 h. T.l.c. (solvent E) then showed that only traces of the starting material ( $R_f$  0.35) were present. The mixture contained mainly the acetates VI and VII ( $R_f$  0.30 and 0.25) and some by-products ( $R_f$  0.1–0.2) were also present. After neutralization with aqueous sodium hydrogen carbonate the solution was extracted with chloroform, the chloroform solution was dried and concentrated. Chromatography (solvent F) on a column of silica gel gave 0.46 g (51%) of the  $\alpha$ -acetate VI,  $[\alpha]_D^{25} + 75^\circ$  (c 1, chloroform), which could not be crystallized.

For  $C_{24}H_{28}O_8$  (444.46) calculated: 64.85% C, 6.35% H; found: 64.66% C, 6.32% H.

Eluted next was the  $\beta$ -acetate VII (47 mg; 5.2%) which crystallized on standing overnight. After crystallization from ethanol VII melted at 75–76°C and had  $[\alpha]_D^{25} + 19^\circ$  (c 1, chloroform).

Found: 64.53% C, 6.27% H.

b) Compound V (6.5 g) was added portionwise at 100°C to a mixture of toluene (13 ml), acetic anhydride (3.25 ml), and fused sodium acetate (3.25 g), and the mixture was stirred for 1.5 h. T.l.c. (solvent A) then showed that the reaction was complete and that products having chromatographic mobility corresponding to VI and VII (see above) were present. After usual work-up p.m.r. spectrum of the crude product showed that the  $\alpha$ - and  $\beta$ -acetates were present in a ratio of 3:7. Crystallization from ethanol, after seeding with VII obtained from the acetylation, gave 4.05 g (56.4%) of the  $\beta$ -acetate, m.p. 75–76°C.

*Methyl 3,4-di-O-acetyl-2-O-(methyl 2,3-di-O-benzyl-4-O-methyl- $\alpha$ - (IX) and - $\beta$ -D-glucopyranosyluronate)- $\beta$ -D-xylopyranoside (X)*

a) Compound VII, or a crude product of acetylation of V (140 mg) was dissolved in dichloromethane containing hydrogen bromide (0.05 g HBr/ml, 16 ml) and the solution was kept at 20°C for 1.5 h. The mixture was concentrated with co-distillation with toluene and after drying at 20°C/13 Pa bromide VIII was obtained in the form of an orange, chromatographically almost pure sirup,  $[\alpha]_D^{25} + 129^\circ$  (c 1, chloroform).

A solution of methyl 3,4-di-O-acetyl- $\beta$ -D-xylopyranoside [8] (600 mg; 2.4 mmoles) in benzene—nitromethane (1:1; 60 ml) was evaporated at atmospheric pressure until approxi-

mately 20 ml of the solvent mixture had distilled, and then it was cooled to room temperature. Mercuric cyanide (800 mg; 3.15 mmoles) and a solution of VIII [prepared from 1.4 g (3.15 mmoles) of a mixture of acetates VI and VII as described above] in a minimum of benzene were added and the stirred mixture was kept at 20°C for 1 h. T.l.c. (solvent A) showed the absence of VIII ( $R_f$  0.8), the presence of some unaltered methyl 3,4-di-*O*-acetyl- $\beta$ -D-xylopyranoside ( $R_f$  0.1) and of V ( $R_f$  0.3) formed by hydrolysis of VIII. The main reaction products IX and X had  $R_f$  0.5 and 0.4, respectively. The mixture was filtered, the filtrate concentrated and the solution of the residue in chloroform was washed with aqueous 1 M potassium bromide solution, to remove mercuric salts. The product in the chloroform solution, after concentration, was eluted from a silica gel column (solvent G and A) to give first IX (0.62 g, 40.5%, based on the amount of methyl 3,4-di-*O*-acetyl- $\beta$ -D-xylopyranoside), m.p. 110.5—111.5°C (from ether),  $[\alpha]_D^{25} + 56.7^\circ$  ( $c$  1, chloroform).

For  $C_{32}H_{40}O_{13}$  (632.64) calculated: 60.75% C, 6.37% H; found: 60.70% C, 6.40% H.

Subsequently eluted was the oligosaccharide X (0.43 g; 28.1%), m.p. 84—86°C (from ether),  $[\alpha]_D^{25} + 1.5^\circ$  ( $c$  2.75, chloroform).

Found 60.63% C, 6.29% H.

b) Ester V (0.5 g) was dissolved in freshly distilled thionyl chloride (5 ml) and the solution was concentrated after 24 h at 20°C. After several co-distillations with toluene, to remove all volatile products, the residue was extracted with dry ether to give, after concentration, almost pure (t.l.c.) anomeric chlorides XV. P.m.r. spectrum of the product having  $[\alpha]_D^{25} + 45^\circ$  showed that the  $\alpha$ - and  $\beta$ -halides were present in an approximate ratio of  $\alpha:\beta = 1:3.5$  (the spectrum was interpreted with the aid of the spectrum of VIII).

A solution of silver perchlorate (1.66 g; 8 mmoles) in dry ether (180 ml) was added quickly at -10°C to the solution of XV [obtained from V (3.21 g; 8 mmoles)], *sym*-collidine (1.06 ml; 8 mmoles) and methyl 3,4-di-*O*-acetyl- $\beta$ -D-xylopyranoside (0.992 g; 4 mmoles) in dry ether, and the mixture was stirred at 0°C with the exclusion of moisture for 1 h. T.l.c. [*c.f.* (a)] showed the absence of XV, the presence of small amounts of V and of unreacted methyl 3,4-di-*O*-acetyl- $\beta$ -D-xylopyranoside, together with X and mainly of the  $\alpha$ -linked oligosaccharide IX. Small amounts of the products of decomposition of XV (not further examined) had  $R_f \sim 0.6$ . The mixture was filtered, the solids washed with ether and the filtrate, combined with the washings was concentrated. Chromatography of the residue gave 2.17 g (85.8%) of IX and 0.12 g (4.75%) of X (the yields are calculated on the basis of the amount of methyl 3,4-di-*O*-acetyl- $\beta$ -D-xylopyranoside).

*Methyl 3,4-di-O-acetyl-2-O-(methyl 2,3-di-O-acetyl-4-O-methyl- $\alpha$ -D-glucopyranosyluronate)- $\beta$ -D-xylopyranoside (XI)*

a) A mixture of IX (3 g) and 5% palladium on charcoal catalyst (0.5 g) in methanol (150 ml) was stirred at 20°C in a hydrogen atmosphere until t.l.c. (solvent A) showed that the reaction was complete. The product ( $R_f$  0.2; solvent H) was isolated in the usual manner, and acetylated in pyridine (3 ml) with acetic anhydride (3 ml). Isolation in the usual way gave XI as the sole product (t.l.c.,  $R_f$  0.3, solvent A, 2.1 g, 82.5%) which was crystallized from methanol, m.p. 201—203°C,  $[\alpha]_D^{25} + 89^\circ$  ( $c$  1, chloroform).

For  $C_{22}H_{32}O_{15}$  (536.88) calculated: 49.25% C, 6.01% H; found: 49.18% C, 6.11% H.

b) Acetic anhydride (1 ml) was added to a solution of *XII* (0.5 g) in pyridine (2 ml) and the mixture was left at 20°C for 24 h. The product *XI* (0.65 g; 89%) was isolated in the usual way and crystallized from methanol, m.p. 201–202°C,  $[\alpha]_D^{22} + 90.5^\circ$  (c 1, chloroform). Its mass spectrum was identical [25] with that of the substance obtained by conversions of the compound isolated from a natural source. The i.r. spectrum of *XI* was superimposable with that of the acetate kindly supplied by Professor *Timell*, who obtained the substance by conversions of the aldobiouronic acid [26].

*Methyl 3,4-di-O-acetyl-2-O-(methyl 2,3-di-O-acetyl-4-O-methyl-  
-β-D-glucopyranosyluronate)-β-D-xylopyranoside (XIII)*

Compounds *X* and *XIV* were treated as described in the preparation of *XI*, to give *XIII* in yields of 84 and 88%, respectively, m.p. 107–108°C,  $[\alpha]_D^{22} - 48^\circ$  (c 1, chloroform).

Found: 49.14% C, 6.07% H.

The mass spectrum of *XIII* was qualitatively identical with that of *XI*.

*Methyl 2-O-(methyl-4-O-methyl-α-D-glucopyranosyluronate)-  
-β-D-xylopyranoside (XII)*

a) Methanolic 0.1 M sodium methoxide (2 ml) was added to a solution of *XI* (800 mg) in dry methanol (80 ml) and the reaction mixture was left at 20°C for 30 min. T.l.c. with solvent *H* showed that no mobile components were present; when solvent *I* was used for t.l.c. a single product ( $R_f$  0.3) was detected. The solution was neutralized with Dowex 50 W ( $H^+$ ) resin, filtered and concentrated to give a crystalline residue. Recrystallization from methyl acetate afforded pure *XII* melting at 167–168°C and having  $[\alpha]_D^{25} + 75^\circ$  (c 1, methanol). P.m.r. data ( $\delta$ ): 4.42 (1-proton doublet, H-1,  $J_{1,2}$  7.3 Hz), 3.39 (1-proton triplet, H-2,  $J_{2,3}$  9.5 Hz), 5.25 (1-proton doublet, H-1',  $J_{1',2'}$  3.2 Hz), 3.56 (1-proton quartet, H-2',  $J_{2',3'}$  9.6 Hz), 3.83 (1-proton triplet, H-3',  $J_{3',4'}$  9.5 Hz), 3.33 (1-proton triplet, H-4',  $J_{4',5'}$  10 Hz), 4.66 (1-proton doublet, H-5'), 3.52, 3.46 (two 3-proton singlets,  $2 \times OCH_3$ ), 3.83 (3-proton singlet,  $COOCH_3$ ).

For  $C_{14}H_{24}O_{11}$  (368.33) calculated: 45.65% C, 6.57% H; found: 45.58%, C, 6.40% H.

b) Compound *IX* (2 g) was debenzylated by catalytic hydrogenolysis as described in the preparation of *XI*. When the reaction was complete the catalyst was filtered off and 0.1 M methanolic sodium methoxide (2 ml) was added to the filtrate. After 30 min at 20°C the reaction mixture was worked up in the usual manner and *XII* was crystallized from methyl acetate. The thus obtained material (0.9 g; 77.3%) melted at 166–167°C.

*Methyl 2-O-(methyl-4-O-methyl-β-D-glucopyranosyluronate)-  
-β-D-xylopyranoside (XIV)*

The procedures applied in the preparation of the  $\alpha$ -linked aldobiouronic acid derivative *XII* gave from *X* or *XIII* the deblocked oligosaccharide *XIV* in 81 and 87% yields,

respectively. The substance melted at 209—212°C, had  $[\alpha]_D^{22} -37.5^\circ$  (c 0.8, methanol), and exhibited the following p.m.r. characteristics ( $\delta$ ): 4.41 (1-proton doublet, H-1,  $J_{1,2}$  7.2 Hz), 4.71 (1-proton doublet, H-1',  $J_{1',2'}$  7.4 Hz), 4.06 (1-proton doublet, H-5',  $J_{4',5'}$  10 Hz), 3.47 (6-proton singlet,  $2 \times \text{OCH}_3$ ), 3.86 (3-proton singlet,  $\text{COOCH}_3$ ).

Found: 45.61% C, 6.40% H.

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