Synthesis and reactions of uronic acid derivatives. IV.* Unambiguous synthesis of methyl(methyl α- and β-D-glucopyranosid)uronate

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Optimum conditions for the oxidation of primary hydroxyl groups of carbohydrates to the corresponding uronic acid derivatives with chromium trioxide and dilute sulfuric acid in acetone have been determined using methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranoside as the substrate. Methyl 2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside, methyl 2,3,4-tri-*O*-benzyl-α-D--glucopyranoside, methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranoside, and methyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside were oxidized under the most favourable conditions and the formed uronic acid derivatives were esterified with diazomethane. Removal of the blocking groups from the produced methyl uronates afforded methyl(methyl α- and β-D-glucopyranosid)uronate of which the latter has been for the first time obtained crystalline.

D-Glucuronic acid is a substance of considerable biological importance. In nature it occurs as an important constituent of metabolic products of plants and animals. Although in these substances D-glucuronic acid is present mainly in the pyranose form, its synthetic pyranose derivatives are not readily obtainable because, in contrast to D-galacturonic acid [1—3], D-glucuronic acid shows a pronounced tendency to lactonize and it enters most of the reactions in its furanose form.

Oxidation of 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose followed by deacetylation gave D-glucuronic acid [4] which spontaneously lactonized and was isolated as D-glucofuranurono(6 → 3)-lactone, commonly known as D-glucuronolactone or D-glucurone. Similarly, other procedures of preparation of D-glucuronic acid [5, 6] gave in the final step the more stable D-glucurone. Treatment of D-glucurone with methanolic hydrogen chloride at room temperature [7] afforded γ-lactone of methyl β-D-glucofururonoside. This by further treatment with the same reagent under more vigorous conditions gave a syrupy mixture of glycosides from which no substance could be isolated in the anomerically pure state. It follows from the foregoing that a synthesis of anomerically pure derivatives of D-glucopyranuronic acid has to be done by a roundabout way *via* precursors having the pyranose structure fixed by suitable substitution.

* For Part III see Ref. [22].

The present paper describes practical procedures for making methyl ester methyl glycosides of D-glucopyranosiduronic acid by the oxidation of primary hydroxyl group of methyl α- and β-D-glucopyranoside having the secondary positions protected with removable substituents.

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 were recorded at 80 MHz with a Tesla BS 487 B spectrometer for solutions in chloroform-*d* (if not otherwise stated), with tetramethylsilane as the internal standard. The proton-signal assignments were made by the INDOR technique. Thin-layer chromatography (t.l.c.) was performed on Silica Gel G coated glass slides and column chromatography on dry-packed silica gel [32] (0.05—0.1 mm) columns with: *A*. *n*-hexane—ethyl acetate 4 : 1, *B*. benzene—acetone 3 : 1, *C*. *n*-hexane—acetone 10 : 1, *D*. benzene—acetone 10 : 1, *E*. benzene—acetone 25 : 1, *F*. benzene—acetone 50 : 1, and *G*. chloroform—methanol 6 : 1. The solvent ratios are based on volumes. Detection was by charring with 5% sulfuric acid in ethanol until permanent spots were visible. Olefinic components were located by spraying with fresh 0.1% potassium permanganate in acetone with no heating applied. Oxidized substances appeared immediately as yellow spots on a violet background. 1,2-Dimethoxyethane (DME) was dried as described by *Perrin et al.* [33] and stored over sodium hydride. Acetone and chromium trioxide were commercial products used as supplied. Methyl 2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside was prepared according to the published directions [24]. The compound melted at 143—145°C and had $[\alpha]_D^{20}$ +52° (*c* 1.06, chloroform), $[\alpha]_D^{20}$ +130° (*c* 1.02, pyridine). Ref. [24] m.p. not given, $[\alpha]_D^{17}$ +131.4° (*c* 1, pyridine). Its p.m.r. spectrum showed overlapped signals (doublet and quartet) for H-1 and H-2 in the region of δ 5.43—5.20. The signals for other protons appeared at δ 6.27 (1-proton octet, H-3), δ 5.59 (1-proton doublet, H-4), δ 4.04 (1-proton doublet of doublets, H-5), δ 3.76 (2-proton doublet of doublets, CH_2), δ 3.05 (1-proton singlet which disappeared on deuteration, OH), δ 3.44 (3-proton singlet, OCH_3), δ 7.5 (complex multiplet, aromatic protons).

Uronoamides were prepared by amonolysis [21] of the corresponding methyl esters. The solutions were concentrated under diminished pressure at <40°C.

Methyl 2,3,4-tri-O-methyl-6-O-trityl-α-D-glucopyranoside (I)

Sodium hydride (12 g) was added portionwise to a chilled solution of methyl 6-*O*-trityl-α-D-glucopyranoside [24] (22 g) in DME (300 ml) followed by an addition of methyl iodide (20 ml). The mixture was stirred and heated gently with the exclusion of atmospheric moisture and carbon dioxide in the reaction flask equipped with an efficient stirrer and condenser. Heating was terminated when a rather vigorous reaction was noticeable and the mixture was stirred until the reflux ceased (approximately 1 hr), and then t.l.c (solvent *A*) showed only one product (R_F 0.4). (When an inefficient condenser is used some methyl iodide may escape from the reaction mixture resulting in incomplete methylation indicated by the presence of several slower moving components. The reaction may be completed, now without heating, by addition of methyl iodide and stirring until t.l.c. shows the absence of any undermethylated product.) The excess of the methylation agent was decomposed by careful addition of methanol and when the

evolution of hydrogen ceased the mixture was transferred into a round-bottom flask, diluted with an equal amount of water und the organic solvents were evaporated. The water phase was extracted with chloroform and the latter backwashed with water until neutral, dried with anhydrous sodium sulfate and concentrated to give syrupy crude *I* (24 g, ~ 100%) sufficiently pure for the next step. A portion was recrystallized from *n*-hexane (twice) to give chromatographically pure substance *I* having m.p. 107—108°C, $[\alpha]_D^{25}$ +97° (*c* 1, acetone) which did not change on recrystallization from either chloroform — *n*-hexane or ethanol. Compound *I* is apparently dimorphous as the values reported [34] are: m.p. 166—167°C and $[\alpha]_{5461}^{24}$ + 88.8° (*c* 0.76, acetone). I.r. spectrum of *I* showed no hydroxyl absorption. P.m.r. spectrum showed the presence of 15 aromatic protons at δ 7.29. Other definite signals were at δ 4.91 (1-proton doublet, H-1, $J_{1,2}$ 3.5 Hz), δ 3.07 (1-proton doublet of doublets, H-2), δ 3.28, 3.42, 3.54, and 3.61 (four 3-proton singlets, OMe), δ 3.64—3.25 (complex multiplet of H-3—H-5 and H-6 and H-6').

For $C_{29}H_{34}O_6$ (478.56) calculated: 72.78% C, 7.16% H, 25.94% CH_3O; found: 72.77% C, 6.91% H, 26.14% CH_3O.

Methyl 2,3,4-tri-O-methyl-α-D-glucopyranoside (II)

Water was added portionwise to a solution of *I* (10.6 g) in acetic acid (20 ml) at 100°C until faint turbidity and kept at this temperature for 2 hrs at which time t.l.c. (solvent *A*) showed that the reaction was almost complete. The solution was kept overnight at room temperature and the separated noncarbohydrate by-products were filtered. Acetic acid was removed from the filtrate by co-evaporation with water and ethanol, the 6-*O*-acetate (R_F 0.3, solvent *B*) formed spontaneously during evaporation of the solution was deacetylated with sodium methoxide in methanol, and the deionized solution (Dowex 50W (H^+)) was concentrated. Pure *II* was obtained by elution of the residue from a silica gel column with solvent *B* and vacuum distillation (b.p. 120—140°C (bath)/0.05 torr). The colourless oil (4.7 g, 89.7%) had $[\alpha]_D^{23}$ +160° (*c* 1, methanol). Ref. [34] $[\alpha]_{5461}^{20}$ +175.3° (*c* 1, methanol). Definite signals in the p.m.r. spectrum of *II* were at δ 4.81 (1-proton doublet, H-1), δ 3.16 (1-proton doublet of doublets, H-2, $J_{1,2}$ 3.5 Hz), δ 2.22 (1-proton singlet which disappeared on deuteration, OH), and at δ 3.43, 3.52, 3.56, and 3.63 (four 3-proton singlets, OMe).

Methyl 2,3,4-tri-O-benzyl-6-O-trityl-α-D-glucopyranoside (IV)

Sodium hydride (10 g) was added portionwise to a stirred solution of methyl 6-*O*-trityl-α-D-glucopyranoside [24] (18 g) in DME (280 ml) at 0°C followed by addition of benzyl bromide (42.5 ml). The mixture was heated under gentle reflux and stirred with the exclusion of atmospheric moisture and carbon dioxide for 8 hrs and left overnight at room temperature. T.l.c. (solvent *C*) showed then the presence of a single product (R_F 0.5). The reaction mixture was processed as described in the preparation of *I*, benzyl ether and benzyl methyl ether were removed by vacuum distillation at 140°C (bath)/0.05 torr to give a product (30 g, ~100%) which was sufficiently pure for the next step. The analytical sample of *IV* (purified by chromatography) had $[\alpha]_D^{24}$ +17.7° (*c* 1, chloroform).

For $C_{47}H_{46}O_6$ (706.84) calculated: 79.86% C, 6.56% H, 4.39% CH_3O; found: 79.79% C, 6.54% H, 4.17% CH_3O.

Methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside (V)

The crude *IV* (25 g) was detritylated with dilute acetic acid and the reaction mixture was worked up as described in the preparation of *II*. Final purification by column chromatography (subsequent elution with benzene and solvent *D*) gave pure, syrupy *V*, having $[\alpha]_D^{27}$ +24° (*c* 1, chloroform)*. P.m.r. spectrum showed the presence of 15 aromatic protons at δ 7.27, the methyl protons appeared as a singlet at δ 3.23 and the broad singlet of OH (disappeared on deuteration) was at δ 2.59.

For $C_{28}H_{32}O_6$ (464.54) calculated: 72.79% C, 6.94% H, 6.68% CH_3O; found: 72.10% C, 6.81% H, 6.90% CH_3O.

Methyl 2,3,4-tri-O-benzyl-β-D-glucopyranoside (XII)

Benzylation under the conditions described for the preparation of the α anomer of methyl 6-*O*-trityl-β-D-glucopyranoside [24] (10 g) gave a crude product which was detritylated with dilute acetic acid and after processing the crude material as described above, the product *XII* was crystallized from cyclohexane. Chromatographically pure *XII* thus obtained melted at 76—83°C (8.5 g, 80%). A portion was recrystallized from 70% ethanol and the melting point observed then was 89—91°C. Ref. [25] gives m.p. 90—91°C.

Methyl(methyl 2,3,4-tri-O-methyl-α-D-glucopyranosid)uronate (III)

To a solution of *II* (1 g) in acetone (16 ml) a solution of chromium trioxide in 3.5 M-H_2SO_4 (50, 100, and 200% excess according to equation (*1*) was added portionwise and with stirring at 0°C. Cooling was terminated after 10 min and the mixture was stirred at room temperature for additional 15, 30, 60, and 120 min, filtered through a sintered-glass filter-funnel of medium porosity onto crushed ice (50 g). The solids were washed with acetone until the filtrate was colourless and the combined filtrate and washings were concentrated to remove the organic solvent. The aqueous phase was washed thoroughly with chloroform which was then backwashed with a little water, dried with anhydrous sodium sulfate, and evaporated to dryness. The residue in a little methanol was added to the top of a column of freshly prepared (washed subsequently with freshly distilled water and methanol) Amberlite IR-402 (OH^-) resin (twofold excess). Elution with methanol removed some unchanged starting material and oxidation by-products (monitoring of evaporated 50 ml fractions of the eluate by t.l.c.) and the free acid was obtained by elution with methanol—acetic acid—water (45:45:10, ten bed volumes). The eluate was concentrated with periodical additions of water to remove acetic acid, the residue was dissolved in a little methanol and excess of ethereal diazomethane was added. The resulting methyl ester *III* was purified by column chromatography (solvent *E*) and weighed. The yields are summarized in Table 1. After vacuum distillation (b.p. 120°C (bath)/0.02 torr) compound *III* had $[\alpha]_D^{25}$ +156° (*c* 2.05, methanol). Ref. [35] $[\alpha]_D$ +156° (*c* 2, methanol), Ref. [17] $[\alpha]_D^{30}$ +153° (*c* 1, water). The derived methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosiduronamide melted at 187—188°C. Ref. [35] m.p. 188—189°C.

* Note added in proof: While the manuscript of this paper was under editorial processing, a procedure appeared according to which this compound could be obtained in crystalline condition [*Carbohyd. Res.* ***34***, 79 (1974)]. Our preparation readily crystallized from cold isopropyl ether and its physical properties closely coincided with the data given here, when seeded with the material kindly supplied by the authors of the quoted work. The gift of the seed crystals is hereby gratefully acknowledged.

Methyl(methyl 2,3,4-tri-O-benzyl-α-D-glucopyranosid)uronate (VI)

A solution of chromium trioxide (2.6 g) in 3.5 M-H_2SO_4 (11 ml) was added slowly to compound *V* (4.5 g) in acetone (72 ml) at 0°C. Cooling was terminated 10 min after the addition was complete and after additional 60 min the reaction mixture was worked up as described for preparation of *III*. Separation of the acidic product on the anion exchanger, esterification with ethereal diazomethane and final purification of the produced methyl ester on a silica gel column afforded 3.9 g (81.7%) of pure *VI* which had, after vacuum distillation (b.p. 140–145°C/0.01 torr), $[\alpha]_D^{27}$ +13° (*c* 1.04, chloroform). P.m.r. data (δ): 4.13 (1-proton doublet, $J_{1,2}$ 3.5 Hz, H-1), 3.57 (1-proton doublet of doublets, H-2), 3.73 (3-proton singlet, COOMe), 3.40 (3-proton singlet, OMe), 7.27 (multiplet of aromatic protons).

For $C_{29}H_{32}O_7$ (492.55) calculated: 70.71% C, 6.55% H, 12.61% CH_3O; found: 70.46% C, 6.50% H, 12.77% CH_3O.

The derived methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranosiduronamide had m.p. 166–167°C (from methanol, twice) and $[\alpha]_D^{24}$ +29° (*c* 1.03, methanol). P.m.r. data (δ): 4.59 (1-proton doublet, $J_{1,2}$ 3.5 Hz H-1), 3.52 (1-proton doublet of doublets, H-2), 3.33 (3-proton singlet, OMe), 6.25 (two proton doublet, NH_2, changed to a singlet at 50°C), 7.25 (multiplet of aromatic protons).

For $C_{28}H_{31}O_6N$ (477.54) calculated: 70.42% C, 6.54% H, 2.93% N; found: 70.47% C, 6.50% H, 2.77% N.

Methyl(methyl 2,3,4-tri-O-benzoyl-α-D-glucopyranosid)uronate (VIII)

Methyl 2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside (10 g) was oxidized under optimum conditions in acetone (160 ml) with chromium trioxide (5.27 g) in 3.5 M sulfuric acid (22.6 ml). The reaction mixture was filtered onto ice (350 g), the solids washed with acetone and from the combined filtrate with the washings the organic solvents were removed. The water phase from which some oily product started to separate was extracted with chloroform, the chloroform solution backwashed with water, dried, and concentrated to give a crude product (11.2 g) as a solid foam. This was divided into two equal portions one of which was debenzoylated [see below for the preparation of *VII* (C)]. The other portion, containing mainly methyl 2,3,4-tri-*O*-benzoyl-α-D-glucopyranosiduronic acid, was esterified in a little methanol by addition of an excess of ethereal diazomethane. T.l.c. in solvent *E* showed then the presence of minimum by-products, some unaltered starting material (R_F 0.2) and methyl ester *VIII* (R_F 0.6). Chromatography of this material gave then 4.2 g (79.5%) of pure *VIII* as a solid foam. The yield given here is based on 5 g of the starting material. Compound *VIII* had $[\alpha]_D^{22}$ +62° (*c* 0.97, chloroform). P.m.r. data (δ): 5.38 (1-proton doublet. $J_{1,2}$ 2.5 Hz, H-1), 5.34 (1-proton doublet of doublets, H-2), 6.25 (1-proton multiplet, H-3), 5.67 (1-proton doublet of doublets, H-4), 4.60 (1-proton doublet, H-5), 3.66 (3-proton singlet, COOMe), 3.46 (3-proton singlet, OMe), 7.6 (complex multiplet, aromatic protons), $J_{4,5}$ 9.5 Hz.

For $C_{29}H_{26}O_{10}$ (534.50) calculated: 65.16% C, 4.90% H, 11.61% CH_3O; found: 65.05% C, 4.75% H, 11.72% CH_3O.

The crude product of amonolysis of *VIII* was partitioned between water and ether to remove methyl benzoate and the water phase was concentrated. The solid residue was crystallized from ethanol to give pure methyl α-D-glucopyranosiduronamide (silky needles) which, when dried at 100°C/15 torr, melted at 170–171°C and had $[\alpha]_D^{22}$ +152° (*c* 1.5, methanol). When the same substance was dried at 40°C/15 torr, it had eventually

the same physical constants. However, a change of the crystalline configuration could be observed on melting point determination. At 140—150°C the stick-like crystals gradually changed to silky needles which then melted at 170—171°C. Ref. [10] gives m.p. 168°C and $[\alpha]_D$ +135° (*c* 1, methanol).

For $C_7H_{13}O_6N$ (207.18) calculated: 40.58% C, 6.32% H, 6.76% N; found: 40.53% C, 6.38% H, 6.80% N.

Methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosiduronic acid (IX) and its methyl ester (X)

Methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranoside [27] (3.7 g) was oxidized in acetone (65 ml) with a solution of chromium trioxide (3.05 g) in 3.5 M sulfuric acid (13.2 ml) and the mixture was worked up as described above. The crude product (3.8 g) obtained on concentration of the chloroform extracts was dissolved in methanol (10 ml) and a little ether was added. Some amorphous material was removed by filtration through medium porosity sintered-glass funnel and the filtrate was concentrated. The acid *IX* was then obtained (3 g, 78.4%) by crystallization from chloroform—ether and had after repeated crystallization from the same solvent m.p. 128—128.5°C and $[\alpha]_D^{24}$ −22° (*c* 1, chloroform). P.m.r. data (δ): 4.54 (1-proton doublet, $J_{1,2}$ 7.0 Hz, H-1), 4.99 (1-proton doublet of doublets, H-2), 4.03 (2-proton multiplet, H-4 and H-3), 3.52 (3-proton singlet, COOMe), 2.04 (9-proton singlet, COMe), 11.69 (1-proton singlet, COOH).

For $C_{13}H_{18}O_{10}$ (334.27) calculated: 46.71% C, 5.43% H, 9.28% CH_3O; found: 46.66% C, 5.07% H, 8.99% CH_3O.

Methyl(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate *X* was obtained in almost theoretical yield by esterification of the acid *IX* with ethereal diazomethane. When crystallized from methanol compound *X* melted at 154—156°C (sublimation). Ref. [36] gives m.p. 151—152°C (sublimation), Ref. [11] gives m.p. 153°C.

Methyl(methyl 2,3,4-tri-O-benzyl-β-D-glucopyranosid)uronate (XIV)

Compound *XII* (3.4 g) in acetone (55 ml) was oxidized with chromium trioxide (1.95 g) and 3.5 M sulfuric acid (8.4 ml) in the manner described in the preparation of *VI*. Work-up and elution of the free acid from the anion-exchange resin gave after evaporation a crystalline residue containing mainly the free acid *XIII*. It was dissolved in a little methanol and an excess of ethereal diazomethane was added to give the title ester *XIV* which crystallized immediately on concentration. Recrystallization from methanol yielded chromatographically pure *XIV* (2.6 g, 72.2%, based on the starting compound *XII*), m.p. 96—97°C, $[\alpha]_D^{24}$ −1° (*c* 1.05, chloroform). P.m.r. data (δ): 4.35 (1-proton doublet, $J_{1,2}$ 7.1 Hz, H-1), 3.69 (3-proton singlet, COOMe), 3.53 (3-proton singlet, OMe), 7.2 (second order multiplet, aromatic protons).

For $C_{29}H_{32}O_7$ (492.55) calculated: 70.71% C, 6.55% H, 12.60% CH_3O; found: 70.48% C, 6.54% H, 12.40% CH_3O.

Methyl(methyl α-D-glucopyranosid)uronate (VII)

A. Compound *VI* (2.1 g) dissolved in methanol was hydrogenated at room temperature and atmospheric pressure in the presence of palladium-on-charcoal catalyst (0.5 g) until t.l.c. in solvent *G* showed that the reaction was complete. The solution was filtered, concentrated, and the residue dried at 30°C/15 torr to give a colourless syrup (943 mg,

~ 100%) which had $[\alpha]_D^{22}$ +136°C (*c* 1, methanol). Ref. [10] gives no physical constants for the dark syrup described. P.m.r. data (δ) for the solution of *VII* in acetone-d_6: 4.7 (1-proton doublet, $J_{1,2}$ 3.3 Hz, H-1), 3.74 (3-proton singlet, COOMe), 3.42 (3-proton singlet, OMe).

For $C_8H_{14}O_7$ (222.19) calculated: 43.21% C, 6.35% H, 27.93% CH_3O; found: 43.07% C, 6.41% H, 27.74% CH_3O.

The corresponding amide melted at 170—171°C and was in all other respects identical with the amide obtained by the amonolysis of *VIII*.

By conventional procedure, compound *VII* could be converted into methyl(methyl 2,3,4-tris-*O*-*p*-nitrobenzoyl-α-D-glucopyranosid)uronate melting at 173—175°C. The optical rotation observed for this compound was $[\alpha]_D^{22}$ +41° (*c* 1, chloroform). P.m.r. data (δ): 5.33—5.43 (1-proton doublet overlapped with 1-proton quartet, H-1 and H-2, respectively, $J_{1,2}$ 3.5 Hz), 6.34 (1-proton doublet of doublets, H-3), 5.78 (1-proton doublet of doublets, H-4), 4.68 (1-proton doublet, H-5), 3.71 (3-proton singlet, COOMe), 3.56 (3-proton singlet, OMe), 8.06 (second order multiplet, aromatic protons).

For $C_{29}H_{23}O_{16}N_3$ (669.49) calculated: 52.02% C, 3.46% H, 6.28% N; found: 51.85% C, 3.65% H, 6.14% CH_3O.

B. Water was added dropwise and with stirring into a solution of *VIII* (2 g) in DME (20 ml) until faint turbidity persisted, followed by 10% water solution of sodium hydroxide until the solution was strongly alkaline to litmus. The mixture was left at room temperature overnight, deionized with Dowex 50W (H^+), filtered and the organic solvent was removed. More water was added and the solution was extracted with ether to remove benzoic acid. Concentration left a syrup which was dissolved in methanol and esterified with ethereal diazomethane. T.l.c. in solvent *G* then showed the presence of two main products the major of which moved at the same rate as *VII* (R_F 0.5) obtained as described above. The second product immediately reduced potassium permanganate and had the same chromatographic mobility (R_F 0.6) as a standard sample [28] of methyl(methyl 4-deoxy-β-L-*threo*-hex-4-enopyranosid)uronate. The olefinic sugar, together with other minor by-products, was removed by elution from a small silica gel column and *VII* was finally dried at 30°C/15 torr. The colourless syrup (0.69 g, 83%) had $[\alpha]_D^{22}$ +129° (*c* 1.3, methanol).

C. The crude product of the oxidation of methyl 2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside (5.6 g) corresponding to 5 g of the starting material (see above) was debenzoylated as described in *B*. The major reaction product purified by chromatography had $[\alpha]_D^{24}$ +130° (*c* 1.2, methanol).

The substances obtained *via* the procedures B and C gave amides identical with those obtained by amonolysis of *VII* (procedure A) or *VIII*.

Methyl(methyl β-D-glucopyranosid)uronate (XI)

A. The acid *IX* (1 g) was deacetylated as described for the preparation of *VII* (procedure C). After deionization of the reaction mixture with Dowex 50W (H^+) the solution was concentrated with periodical addition of water to remove some acetic acid, and the semisolid residue dissolved in a little methanol was treated with ethereal diazomethane. T.l.c. of the crude product in solvent *G* showed that some material which immediately reduced potassium permanganate was also present. It moved at the same rate as an authentic sample of methyl(methyl 4-deoxy-α-L-*threo*-hex-4-enopyranosid)uronate (R_F 0.6) [28]. Pure *XI* (0.5 g, 75.3%) was obtained by elution from a silica gel column. The syrup obtained on concentration solidified on standing and after recrystallization

from butanone (twice) melted at 97.5—98.5°C and had $[\alpha]_D^{22}$ −55° (*c* 1, methanol). P.m.r. data (δ) for the solution of *XI* in acetone-d_6: 4.70 (1-proton doublet, $J_{1,2}$ 7.5 Hz, H-1), 3.27 (doublet of doublets, H-2), 3.75 (3-proton singlet, COOMe), 3.46 (3-proton singlet, OMe).

For $C_8H_{14}O_7$ (222.19) calculated: 43.21% C, 6.35% H, 27.93% CH_3O; found: 43.04% C, 6.11% H, 28.0% CH_3O.

Amonolysis of *XI* gave methyl β-D-glucopyranosiduronamide melting at 199—201°C (sublimation). Ref. [36] gives m.p. 198—200°C.

B. The ester *X* was deacetylated as described in the preparation of *VII* (procedure B). Compound *XI* (0.5 g, 78.6%), obtained after purification of the crude product by chromatography melted at 97—99°C and was in all respects identical with the above-described substance.

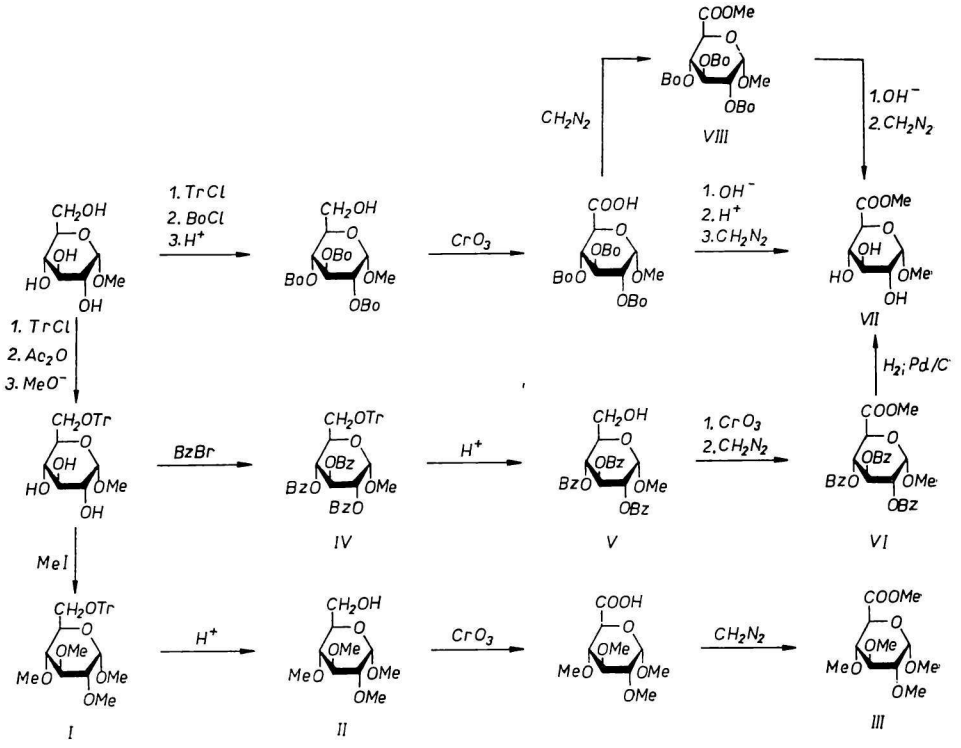
C. The benzyl derivative *XIV* (1 g) was hydrogenated in methyl acetate (30 ml) at room temperature and atmospheric pressure in the presence of 10% palladium-on-charcoal catalyst and when t.l.c. (solvent *G*) showed that the reaction was complete the single product formed (R_F 0.5) was isolated as described for the preparation of *VII* (procedure A) and crystallized from butanone, m.p. 98—99°C, $[\alpha]_D^{25}$ −54° (*c* 1, methanol).

Results and discussion

In connection with the study of the formation of olefinic substances during methylation of uronic acid derivatives with various methylation agents [8] a need arose for larger amounts of methyl(methyl α- and β-D-glucopyranosid)uronate. Because of the reasons mentioned above this class of derivatives must be prepared from suitable derivatives of D-glucose. A number of procedures based on this approach has been described in the literature starting mostly from methyl D-glucopyranosides. *Assarson* and *Theander* [9] oxidized methyl α- and β-D-glucopyranoside with liquid nitrogen tetraoxide and showed, by isolation of four theoretically possible ketoglycosides, in addition to a large amount of oxidative demethylation product, the impropriety of this oxidation agent as far as the oxidation of primary hydroxyl groups of otherwise unsubsituted glycosides is concerned. Their results explain at the same time the failure of previous workers [10, 11] to isolate pure methyl(methyl α- and β-D-glucopyranosid)uronates from the reaction mixtures of oxidation with nitrogen tetraoxide and subsequent esterification of methyl α- and β-D-glucopyranoside. In the case of the α anomer the cited authors [10] isolated low yield of a product in the form of brown syrup for which they gave neither physical constants nor analytical figures. This crude material was acetylated and amonolyzed to yield crystalline methyl α-D-glucopyranosiduronamide which gave analytical data consistent with this structure. In the case of the β anomer [11] a low yield of dark syrup was obtained which could not be induced to crystallize. Acetylation of 16 g of such a product gave only 550 mg of pure methyl(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosid)uronate and this could be converted into crystalline methyl β-D-glucopyranosiduronamide. It is quite obvious that methyl(methyl α- and β-D-glucopyranosid)uronates of the previous authors [10, 11] were not pure substances. Apparently, the preparation of this class of compounds has to be carried out either by oxidation of unprotected glycosides of neutral saccharides under milder reaction conditions or precautions must be made against the formation of unwanted by-products using protecting groups removable in the final stage of the synthesis. The conditions of catalytic oxidation of primary hydroxyl group of carbohydrates with oxygen [12] have been found sufficiently mild but the yields of the desired products obtained by this procedure

have been sometimes low [13] and occasionally [14, 15] oxidation has been unsuccessful. The two-step procedure [16—18] according to which a primary hydroxyl group is first oxidized to an aldehyde which is then oxidized to carboxylic acid widens the choice of oxidation procedures for making uronic acid derivatives from glycosides. Chromium trioxide—dilute sulfuric acid method has also been found quite satisfactory in the oxidation of the primary hydroxyl groups of carbohydrates. By choosing appropriate reaction conditions, high yields of ω-aldehydo carbohydrate derivatives [19] or uronic acids [15, 20, 21] may be obtained with this oxidation agent. Although in the latter case the reaction is conducted under acidic conditions, carbohydrates bearing acid-labile *O*-isopropylidene groups can also be successfully converted to the corresponding uronic acid derivatives [22].

Variable reaction conditions have been used in the synthesis of uronic acid derivatives by the chromium trioxide—dilute sulfuric acid method. We have determined the optimum conditions as far as the amount of the oxidation agent and the duration of the reaction is concerned. Methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranoside (*II*) used as the substrate in this study was prepared by detritylation of methyl 2,3,4-tri-*O*-methyl-6-*O*-triphenylmethyl-α-D-glucopyranoside (*I*) (Scheme 1). It is known that when carbohydrates bearing the bulky trityl group are methylated by conventional methods [17, 23] good yields of fully sub-

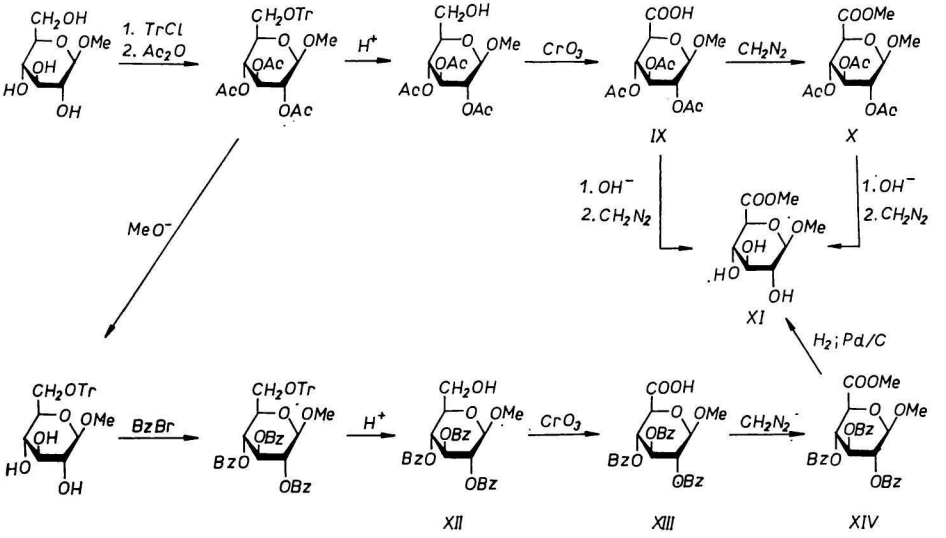


Ac — acetyl, Bo — benzoyl, Bz — benzyl, Me — methyl, Tr — triphenylmethyl.

Scheme 1

stituted products are not obtained. Methylation of methyl 6-*O*-trityl-α-D-glucopyranoside [24] with sodium hydride and methyl iodide in 1,2-dimethoxyethane gave, however, high yield of the wanted substance. Equally good yield was obtained when methyl 6-*O*-trityl-α- and -β-D-glucopyranoside were benzylated with sodium hydride and benzyl bromide using the same procedure.

Methyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside was first prepared and isolated in crystalline condition by *Pravdić* and *Keglević* [25] *via* a sequence more complicated than that described here (1,6-anhydro-β-D-glucopyranose was benzylated, the product acetolyzed to give 1,6-di-*O*-acetyl-2,3,4-tri-*O*-benzyl-β-D-glucopyranose which was subsequently converted into 1-bromo-1-deoxy sugar; condensation with methanol and deacetylation gave then the desired compound). The same authors were also first to convert methyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside into the corresponding crystalline uronic acid derivative by catalytic oxidation. Our product *XIII* also readily crystallized from ethanol. Since it was not a new compound [25] we have not attempted to isolate this substance in an analytically pure state. Instead, in order to obtain the highest possible yield of *XIV* the crude product was esterified to give new, hitherto unknown methyl ester *XIV* (Scheme 2). In view of the good yields of the benzylated compound *XII* from



Scheme 2

methyl 6-*O*-trityl-β-D-glucopyranoside the procedure for making *XII*—*XIV* described herein, allowing the preparation of these compounds without the isolation of the intermediate methyl 2,3,4-tri-*O*-benzyl-6-*O*-trityl-β-D-glucopyranoside is a more convenient one. The steps involved are simple, the sequence does not require the preparation of the unstable bromo sugar and in the oxidation step carried out with chromium trioxide pH-control is not necessary as in the case of catalytic oxidation [12].

As can be seen from Table 1, provided that the course of the oxidation of the model compound *II* can be expressed by the reaction

$$3R{-}CH_2OH + 4CrO_3 + 6H_2O = 3R{-}COOH + Cr_2(SO_4)_3 + 9H_2O \qquad (1)$$

Table 1

Effect of the amount of the oxidation agent upon the conversion of *II* to *III*

Duration [min]	Yield of *III* [%] with the excess of the oxidation agent used		
	50%	100%	200%
15	28	47	63
30	41	70	72
60	64.5	84	78
120	68	79.5	74

the best yield of methyl(methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosid)uronate *III* was obtained by treatment of *II* for 1 hr with a 100% excess of the oxidation agent. When larger excess of the oxidation agent was used or its action was prolonged, the yield of the desired product was lower, probably due to overoxidation (conversion of methyl to formyl derivatives [26]).

When methyl 2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside [24], methyl 2,3,4-tri-*O*-benzyl--α- and -β-D-glucopyranosides, and methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranoside [27] were oxidized under optimum conditions, the yields of the corresponding uronic acid derivatives were comparable with those obtained from *II*.

Methyl(methyl α-D-glucopyranosid)uronate *VII* (colourless syrup) was obtained from *VI* or *VIII* by removal of the blocking groups and purification by chromatography. Its identity with the desired product was proved by its conversion into the known crystalline amide and its structure follows also from its p.m.r. data (see Experimental). Compound *VII* was also characterized by its conversion into crystalline, hitherto unknown per-*O*-*p*-nitrobenzoyl derivative.

Methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosiduronic acid *IX* could be obtained from the reaction mixture of the oxidation of methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranoside by direct crystallization. Esterification with ethereal diazomethane gave the known acetate *X* the melting point of which agreed with the literary value. Deacetylation of *X*, which removed also the methyl ester function, followed by re-esterification afforded crystalline methyl(methyl β-D-glucopyranosid)uronate *XI* which gave the known crystalline amide.

As expected, compounds *VII* and *XI* were obtained in virtually theoretical yields by hydrogenolysis of their precursory per-*O*-benzyl derivatives *VI* and *XIV*. In view of the fact that esterified uronic acid derivatives treated with base under anhydrous conditions easily undergo β-elimination reactions to give 4,5-unsaturated 4-deoxyhexopyranuronates [20, 28] the acetate *IX* and methyl 2,3,4-tri-*O*-benzoyl-α-D-glucopyranosiduronic acid were deacylated before their conversion into the corresponding methyl esters. To avoid possible base-catalyzed esterification [29] which would create the driving force for β elimination the deacylation was carried out in the presence of water and in the absence of an alcohol, rather than, as usual, by an alkoxide in absolute alcohol. The crude reaction mixtures of such deacylation were esterified and examined by t.l.c. which showed that despite the above-mentioned precautions, β elimination had still taken place to some extent. The components of these reaction mixtures which immediately reduced a dilute solution of potassium permanganate had the same chromatographic mobility as standards of methyl(methyl 4-deoxy-α- and β-L-*threo*-hex-4-eno-

pyranosid)uronates. Although the extent of β elimination taking place here was not important from the preparative point of view (the yields of the desired products were not much affected) it is worth noticing that free uronic acid derivatives treated with base even in the presence of water *i.e.* under the conditions unfavourable for β-elimination reactions, may be to some extent degraded by this type of reaction. The decrease of the degree of polymerization of pectic substances during deesterification as a result of β-elimination degradation [30] has been generally recognized. The results of deesterification of the compounds described here, which may be looked upon as models of units of uronic acid-containing polysaccharides suggest that other types of acidic polysaccharides may be structurally altered by β elimination during their extraction from natural sources with water solutions of alkali hydroxides [31].

Examination of the crude reaction mixtures of deacylation of esters *VIII* and *X* carried out with alkali hydroxide in the presence of water showed also the presence of small amounts of unsaturated products. Purification by chromatography on silica gel gave good yields of the title substances *VII* and *XI* of which the β anomer was for the first time obtained crystalline.

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