Synthesis and reactions of uronic acid derivatives. V.* Simple, unambiguous syntheses of methyl(methyl α-D-galactopyranosid)uronate 2-, 3-, and 4-methyl ethers

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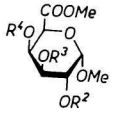
Partial benzoylation of methyl(methyl α-D-galactopyranosid)uronate afforded the three corresponding di-*O*-benzoates. Following methylation under the conditions known not to cause migration of benzoyl groups gave methyl(methyl di-*O*-benzoyl-*O*-methyl-α-D-galactopyranosid)uronates in which the location of methyl groups was determined by mass spectrometry. Debenzoylation yielded the three theoretically possible, all crystalline, monomethyl ethers of methyl(methyl α-D-galactopyranosid)uronate, of which the 3-*O*- and 4-*O*-derivatives were prepared for the first time. Preparation of methyl(methyl 4-deoxy-3-*O*-methyl-β-L-*threo*-hex-4-enopyranosid)uronate is also described.

Methyl ethers of uronic acids are useful for the identification of the products of methylation analysis of acidic polysaccharides and other uronic acid-containing substances. Because of the difficulties involved in the synthesis of suitable intermediates, of the theoretically possible mono-*O*-methyl derivatives of D-galacturonic acid only the 2-methyl ether has been obtained by a regulated synthesis [1, 2].



	R^2	R^3	R^4		R^2	R^3	R^4
I	Bo	H	Bo	*VI*	Me	Bo	Bo
II	Bo	Bo	H	*VII*	H	Me	H
III	H	Bo	Bo	*VIII*	H	H	Me
IV	Bo	Me	Bo	*IX*	Me	H	H
V	Bo	Bo	Me				

Bo — benzoyl, Me — methyl.

Scheme 1

* For Part IV see this issue, p. 820

The present work describes the separation of the three theoretically possible di-*O*-benzoyl derivatives formed on partial benzoylation of methyl(methyl α-D-galactopyranosid)uronate and their use in the synthesis of monomethyl ethers of methyl(methyl α-D-galactopyranosid)uronate.

Experimental

Melting points were determined on a Kofler hot-stage. Optical rotations were measured using a Perkin—Elmer automatic polarimeter Model 141. P.m.r. spectra for the solutions in chloroform-*d* were recorded at 80 MHz on a Tesla BS 487 B spectrometer with tetramethylsilane as the internal standard. Mass spectra were obtained at 70 eV with a MCh-1306 mass spectrometer. The temperature in the site of evaporation was 100°C and in the ionization chamber 160°C. The direct introduction technique was applied. Thin-layer chromatography (t.l.c.) was performed on Silica Gel G and preparative chromatography on dry-packed columns of silica gel using benzene—acetone (*A*. 10 : 1, *B*. 15 : 1, and *C*. 30 : 1) and chloroform—acetone (*D*. 10 : 1 and *E*. 3 : 1) mixtures. Detection was by spraying with 5% sulfuric acid in ethanol and heating until permanent char spots were visible. Olefinic components were located by spraying with 0.1% potassium permanganate in acetone. Boron trifluoride etherate was freshly distilled from calcium hydride. Solutions were concentrated at 15 torr and < 40°.

Methyl(methyl 2,4-di-O-, 2,3-di-O-, and 3,4-di-O-benzoyl-α-D-galactopyranosid)uronate (I—III)

Thin-layer chromatography of partial benzoylation of methyl(methyl α-D-galactopyranosid)uronate [4] (4.8 g) in solvent *A* showed that, in addition to a small amount of the fully benzoylated product (R_F 0.75), three products were present (R_F 0.45, 0.35, and 0.3). The mixture was worked-up and chromatographed on a column of silica gel (120 × 3.6 cm) using solvent *B*. Clean-cut separation was achieved.

First eluted was methyl(methyl 2,3,4-tri-*O*-benzoyl-α-D-galactopyranosid)uronate, m.p. 134—135°C (from methanol—isopropyl ether), 1.35 g (11.7%). Ref [4] gives m.p. 134—135°C.

Next eluted was the 2,4-di-*O*-benzoate *I* (1.26 g, 13.5%) having $[\alpha]_D^{20}$ +140° (*c* 1, chloroform).

For $C_{22}H_{22}O_9$ (430.40) calculated: 61.39% C, 5.15% H, 14.42% CH_3O; found: 61.63% C, 5.26% H, 13.95% CH_3O.

The main reaction product was the known 2,3-di-*O*-benzoyl derivative *II* (5.12 g, 55%), $[\alpha]_D^{20}$ +179° (*c* 1.04, chloroform). Ref. [4] gives $[\alpha]_D$ +172° (*c* 1, chloroform).

The last of the di-*O*-benzoates was the 3,4-di-*O*-benzoyl derivative *III*, $[\alpha]_D^{20}$ +181° (*c* 0.98, chloroform).

Found: 61.24% C, 5.22% H, 14.12% CH_3O.

None of the isomeric dibenzoates could be induced to crystallize.

Methyl(methyl 2,4-di-O-benzoyl-3-O-methyl-α-D-galactopyranosid)uronate (IV)

Compound *I* (0.85 g) dissolved in dichloromethane (15 ml) was cooled in a dry-ice acetone mixture to −20°C and with the aid of an Eppendorf pipette boron trifluoride etherate (0.05 ml) was added with stirring and with the exclusion of atmospheric moisture. Under continued stirring diazomethane in dichloromethane was added dropwise

while temperature of the reaction mixture was kept below −15°C until faint yellow colour persisted. The composition of the reaction mixture was periodically checked by t.l.c. (solvent *B*) and fresh portions of the catalyst (0.01 ml) and diazomethane were added until the ratio between the starting material (R_F 0.2) and the product *IV* (R_F 0.4) remained practically unchanged on further addition of reagents. Small amount of polymethylene, the reaction by-product, was filtered, washed with a little dichloromethane and the filtrate, combined with the washings, was washed with saturated sodium bicarbonate, water, and concentrated. Elution of the residue from a silica gel column (30 × × 2.8 cm) using solvent *C* gave pure *IV* (0.8 g, 91.1%) having m.p. 138—139°C (from ether, twice) and $[\alpha]_D^{20}$ 165° (*c* 1, chloroform).

For $C_{23}H_{24}O_9$ (444.42) calculated: 62.16% C, 5.44% H, 20.9% CH_3O; found: 61.99% C, 5.17% H, 21.03% CH_3O.

Methyl(methyl 2,3-di-O-benzoyl-4-O-methyl- and 3,4-di-O-benzoyl -2-O-methyl-α-D-galactopyranosid)uronate (V and VI)

Methylation of *II* (3.4 g) under the conditions described above gave, after purification of the crude product by chromatography to remove a minor by-product (not further examined), pure 4-*O*-methyl derivative *V* (R_F 0.5, system *B*), 0.9 g (25.6%) having $[\alpha]_D^{20}$ +158° (*c* 1, chloroform). Unchanged starting material (2.5 g) was also recovered.

Found: 62.12% C, 5.25% H, 21.27% CH_3O.

It was observed by monitoring the course of the methylation of *III* (0.3 g) that the conversion of the starting material to the methylated product *VI* (R_F 0.25, system *B*, *c.f.* 0.15 for the starting material) was complete. Elution from a small column of silica gel with solvent *B* removed some coloured material and gave *VI* (0.3 g, ~ 100%) having $[\alpha]_D^{20}$ +191° (*c* 1, chloroform).

Found: 62.01% C, 5.60% H, 20.74% CH_3O.

Methyl(methyl 4-deoxy-3-O-methyl-β-L-threo-hex-4-enopyranosid)uronate (X)

To a solution of *IV* (0.25 g) in a mixture of dry methanol (5 ml) and 2,2-dimethoxypropane (0.5 ml) 1 N sodium methoxide in methanol (1.1 ml) was added with stirring and the reaction mixture was kept at 50°C for 30 min. The reaction mixture was worked-up as described [2] and t.l.c. (system *D*) showed then the presence of one product (R_F 0.3) which immediately reduced a dilute solution of potassium permanganate. The solution was concentrated and the residue was eluted from a silica gel column to remove some coloured material, which then gave pure *X* (117 mg, 95%) having, after drying at 25°C/ /15 torr, $[\alpha]_D^{20}$ +231° (*c* 1.99, methanol). Definite signals in the p.m.r. spectrum of *X* were at (δ): 5.03 (1-proton doublet, $J_{1,2}$ 2.2 Hz, H-1), 6.19 (1-proton doublet, H-4), 3.82 (3-proton singlet, COOMe), 3.48, and 3.56 (two 3-proton singlets, OMe).

For $C_9H_{14}O_6$ (218.20) calculated: 49.54% C, 6.47% H, 42.67% CH_3O; found: 49.90% C, 6.42% H, 42.99% CH_3O.

Methyl(methyl 3-O-Methyl-α-D-galactopyranosid)uronate (VII)

Water was added dropwise and with stirring to a solution of *IV* (1 g) in 1,2-dimethoxyethane (100 ml) until faint turbidity followed by addition of 1 N sodium hydroxide (40 ml) and the solution was left at room temperature for 30 min. T.l.c. (system *E*) showed at this time that no starting material (R_F 0.85) was present and the debenzoylation was completed by keeping the reaction mixture at 50°C for 1 hr. The solution was

deionized with Dowex 50W (H^+), filtered, concentrated and ethereal diazomethane was added to the solution of the residue in a little methanol. T.l.c. of the reaction mixture (solvent *E*) showed that, in addition to a large amount of *VII* (R_F 0.26) some *X* was also present. Purification by chromatography gave pure title substance (415 mg, 78%). M.p. 102—103°C (from butanone-ether), $[\alpha]_D^{22}$ +151° (*c* 0.69, chloroform).

For $C_9H_{16}O_7$ (236.22) calculated: 45.76% C, 6.83% H, 39.41% CH_3O; found: 45.77% C, 6.92% H, 39.22% CH_3O.

Methyl(methyl 4-O-methyl-α-D-galactopyranosid)uronate (VIII)

Debenzoylation of *V* (0.75 g) as described for the preparation of *VII* gave, after purification of the crude product by chromatography 345 mg (86.5%) of pure *VIII*, which, when twice crystallized from acetone, had m.p. 140—141°C and $[\alpha]_D^{22}$ +132.5° (*c* 1, chloroform).

Found: 46.0% C, 7.12% H, 39.68% CH_3O.

Methyl(methyl 2-O-methyl-α-D-galactopyranosid)uronate (IX)

Compound *VI* (70 mg) was debenzoylated in the above-described manner and after chromatography of the crude product on a column of silica gel to remove a small amount of the olefinic by-product having the same mobility on t.l.c. as an authentic sample of methyl(methyl 4-deoxy-2-*O*-methyl-β-L-*threo*-hex-4-enopyranosid)uronate [2], pure *IX* was obtained (28 mg, 75.2%) in the form of a colourless syrup. The syrup crystallized immediately when seeded with methyl(methyl 2-*O*-methyl-α-D-galactopyranosid)-uronate prepared in an independent manner [2] and was in all respects identical with the previously described substance.

Results and discussion

The works by *Gross et al.* [3] have shown that by using diazomethane in the presence of a catalytic proportion of boron trifluoride etherate carbohydrates bearing base-labile substituents can be methylated without migration of these labile groups. Thus, partially acylated carbohydrate derivatives have become attractive intermediates in the syntheses of partially methylated sugars.

Methyl(methyl 2,3-di-*O*-benzoyl-α-D-galactopyranosid)uronate was recently described by *Gill et al.* [4] and we anticipated to use this substance for the preparation of methyl-(methyl 4-*O*-methyl-α-D-galactopyranosid)uronate, which was the original aim of this work. Accordingly, methyl(methyl α-D-galactopyranosid)uronate was benzoylated as described [4]. T.l.c. of the reaction mixture in a solvent system different from the one recommended by the original authors [4] suggested that the two di-*O*-benzoates, isomeric with the major reaction product, namely methyl(methyl 2,3-di-*O*-benzoyl-α-D--galactopyranosid)uronate, may have also been present. The three components were separated on a column of silica gel and obtained in chromatographically pure state. The compounds gave satisfactory analysis for methyl(methyl di-*O*-benzoyl-hexopyranosid)-uronate.

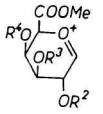
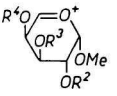
The reactivity of the secondary hydroxyl groups in methyl hexopyranosides was studied by *Williams et al.* [5] who found that the order of reactivity of the secondary hydroxyl groups for an α-D-galacto compound is 2-OH > 3-OH > 4-OH. Taking into account these findings it seemed reasonable to expect that a dimolar benzoylation

of methyl(methyl α-D-galactopyranosid)uronate would result in a reaction mixture containing, in addition to the main product, the 2,3-di-*O*-benzoyl derivative, also the isomeric dibenzoates of which the 2,4-isomer would predominate. Thus the structures *I*—*III* were tentatively assigned to the three isolated di-*O*-benzoates and these were confirmed by mass spectrometry of the corresponding *O*-methyl derivatives *IV*—*VI* which unequivocally determined the position of the methyl groups.

Although benzoyl derivatives of carbohydrates are not considered suitable for investigations by mass spectrometry [6] we have attempted to apply this technique in the determination of the structure of methyl(methyl di-*O*-benzoyl-*O*-methyl-α-D-galactopyranosid)uronates *IV*—*VI*. As expected, the most intense peaks found in the spectra were those of the phenylcarbonium ions at *m/e* 105. Although an analogy for the formation of all the other ions cannot be found among the fragmentations of carbohydrate derivatives studied so far, the spectra contain ions represented by intense peaks (Table 1),

Table 1

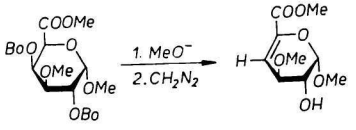
m/e Values of the structurally significant ions found for methyl(methyl di-*O*-benzoyl-*O*-methyl-α-D-galactopyranosid)uronates

Location of OMe group	Symbol of ions	Structure of ions	*m/e*
2			413
3	A_1		413
4			413
2			385
3	E_1		385
4			385
2		$R^4O-\overset{4}{C}H=\overset{3}{C}H-\overset{2}{C}H=\overset{+}{O}R^2$	191
3	F_1		281
4			191
2		$R^3\overset{+}{O}=\overset{3}{C}H-\overset{2}{C}H-OR^2$	178
3	H_1		178
4			—
2		$R^3O-\overset{+}{C}H-OMe$	—
3	J_1		75
4			—

the origin of which has been elucidated in the study of the fragmentation of methyl(methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosid)uronate [7]. Based on the structure of these ions the location of the methyl groups in *IV*—*VI* could be unequivocally determined in the following manner: from the *m/e* values of the A_1 and E_1 ions (terminology introduced by *Kochetkov* [8]), which are the two most intense peaks in the spectra, the molecular weight 444 of *IV*—*VI* was calculated. The spectrum of *IV* contained also peaks of the ions F_1, H_1, and J_1 at *m/e* 281, 178, and 75, respectively, which confirm the pre-

sence of the benzoyl groups at C(2) and C(4) and of the methoxyl group at C(3). The spectrum of *V* contained a pronounced peak of F_1 ions at *m/e* 191, which, together with the fact that the peak of the ions H_1 at *m/e* 178 was not present in the spectrum of this substance, confirms that here the methoxyl group is located at C(4). As the spectrum of *VI* contains both these well pronounced peaks, this compound bears the methoxyl group at C(2). The absence of J_1 ion peaks in the spectra of *V* and *VI* shows further that the migration of the benzoyl group from the position C(3) to C(1), which would be analogous to the formation of the ions at *m/e* 75 occurring in the fragmentation of the 3-*O*-methyl derivative, does not take place here. Although the yields of *IV*—*VI*, of which the 3-*O*-derivative was obtained crystalline, by methylation of the derivatives *I*—*III* were different in each particular case, these were in agreement with the reactivity of the hydroxyl groups present in the sugar moiety. The course of the methylation was monitored by thin-layer chromatography which showed that while the conversion of *III*, bearing the most reactive hydroxyl group to the derivative *VI*, was complete, the benzoate *II* bearing the axially oriented hydroxyl group gave only 25.6% of the wanted *O*-methyl derivative *V* and this conversion could not be increased even with a large excess of the methylation agent.

In view of the fact that the esterified uronic acid derivatives treated with base easily undergo β-elimination degradation, debenzoylation of *IV*—*VI* was carried out in the presence of water. These conditions favour deesterification [9] and, hence, are favourable



IV *X*

for the removal of the driving force for the β elimination. It was observed by monitoring the course of the debenzoylation of 2,3-di-*O*-benzoate *V* by thin-layer chromatography that only traces of products which immediately reduced a dilute solution of potassium permanganate were present in this reaction mixture. Accordingly, compound *VIII* was isolated, after purification of the crude product by chromatography, in an excellent yield. The extent of β-elimination degradation was greater in the case of the debenzoylation of the benzoates *IV* and *VI* bearing in the C(4) position a better leaving group and, consequently, the yields of the final compounds *VII* and *IX* were lower.

The justification of deacylation of esterified uronic acid derivatives in the presence of water is demonstrated by the isolation of virtually quantitative yield of methyl(methyl 4-deoxy-3-*O*-methyl-β-L-*threo*-hex-4-enopyranosid)uronate *X* from the reaction mixture of debenzoylation of the benzoate *IV* conducted under the conditions favourable for β elimination, *i.e.* with sodium methoxide in dry methanol. A synthesis of compound *X* has to our knowledge not been reported. Preparation of methyl 4-deoxy-3-*O*-methyl-β-L-*threo*-hex-4-enopyranosiduronic acid contaminated with the α anomer has been reported by the Japanese authors [10]. The fact that our intermediate *IV* is a crystalline substance secures the anomeric purity of *X*.

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