Base-catalyzed Degradations of Carbohydrates. II. β-Elimination Reactions of 4-*O*-Substituted Methyl (Methyl 2,3-Di-*O*-methyl-β-D-glucopyranosid)uronates[1]

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Received February 21, 1972

The action of various bases on the three hexuronic esters, methyl (methyl 2,3,4-tri-*O*-methyl-β-D-glucopyranosid)uronate (**12**), methyl (methyl 4-*O*-methanesulfonyl-2,3-di-*O*-methyl-β-D-glucopyranosid)uronate (**13**), and methyl (methyl 4-*O*-acetyl-2,3-di-*O*-methyl-β-D-glucopyranosid)uronate (**14**), leading to elimination of the 4-*O*-substituents and formation of methyl (methyl 4-deoxy-2,3-di-*O*-methyl-α-L-*threo*-hex-4-enopyranosid)uronate (**15**) has been studied. It is concluded that effectiveness of 4-*O*-substituents as leaving groups is in the sequence, methanesulfonyloxy > acetoxy > methoxy.

L'action de diverses bases sur trois esters hexuroniques (méthyl tri-*O*-méthyl-2,3,4-β-D-glucopyranoside) uronate de méthyle (**12**), (méthyl *O*-méthanesulfonyl-4 di-*O*-méthyl-2,3-β-D-glucopyranoside) uronate de méthyle (**13**), et le (méthyl *O*-acétyl-4 di-*O*-méthyl-2,3-β-D-glucopyranoside) uronate de méthyle (**14**) qui mène à l'élimination des *O*-substituants en 4 avec formation du (méthyl déoxy-4 di-*O*-méthyl-2,3-α-L-*thréo*-hexèno-4 pyranoside) uronate de méthyle (**15**) a été étudiée. On a conclu que l'ordre d'efficacité des *O*-substituants en 4 en tant que "leaving group" suit la séquence méthanesulfonyloxy > acétoxy > méthoxy.

Canadian Journal of Chemistry, **50**, 2203 (1972)

Neukom (1, 2), Northcote (3, 4), Rees (5, 6), Kiss (7, 8) and their collaborators have shown that esterified hexuronates are susceptible to base-catalyzed degradation with the formation of unsaturated hexuronates and with the exposure of new reducing groups. When reaction is performed in aqueous solution competing ester hydrolysis occurs with loss of the driving force of the carboalkoxy group towards elimination, and only limited degradation is achieved. This difficulty may be partially overcome by using a non-aqueous system such as sodium methoxide in methanol, in which instance the ester function is regenerated by base-catalyzed ester exchange. The value of this approach in obtaining new structural information on polysaccharides has been demonstrated by Rees and co-workers (6) in studies on colanic acid. Nevertheless, the full potential of this type of reaction for the degradation of polysaccharides has not been realized because of the limited solubility of the substrates, unless etherified or esterified, in non-aqueous solvents. 4-*O*-Substituted methyl (methyl 2,3-di-*O*-methyl-β-D-glucopyranosid)-uronates (**12**, **13**, **14**) have been prepared, and the action of bases on these compounds has been studied in order to assess the relative effectiveness of 4-*O*-substituents as leaving groups in the β-elimination reaction. The 4-*O*-acetyl (**14**) and 4-*O*-methyl (**12**) derivatives provide models for the behavior of D-glucuronic acid end groups in acetylated or methylated polysaccharides.

The well-known methyl (methyl 2,3,4-tri-*O*-methyl-β-D-glucopyranosid)uronate (**12**) was most satisfactorily prepared from methyl 6-*O*-trityl-β-D-glucopyranoside (**1**) by successive methylation, de-*O*-tritylation, oxidation with chromium trioxide and sulfuric acid in acetone (9), and esterification with diazomethane. The other hexuronates were prepared from methyl 2,3-di-*O*-methyl-6-*O*-trityl-β-D-glucopyranoside (**3**). Thus, the trityl ether (**3**) was mesylated, de-*O*-tritylated, oxidized with chromium trioxide and sulfuric acid in acetone, and esterified with diazomethane to give methyl (methyl 4-*O*-mesyl-2,3-di-*O*-methyl-β-D-glucopyranosid)uronate (**13**). A similar sequence of reactions furnished methyl (methyl 4-*O*-acetyl-2,3-di-*O*-methyl-β-D-glucopyranosid)uronate (**14**).

Treatment of methyl (methyl 4-*O*-mesyl-2,3-di-*O*-methyl-β-D-glucopyranosid)uronate (**13**) with sodium methoxide in methanol or ethanolic potassium hydroxide, as described by Kiss (10) for the preparation of related unsaturated

[1]Taken from the M.Sc. thesis of P. E. Barron, Trent University, 1972.

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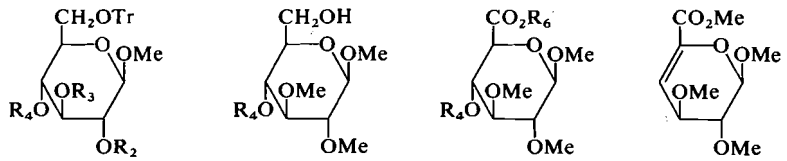

1 $R_2 = R_3 = R_4 = H$
2 $R_2 = R_3 = R_4 = Me$
3 $R_2 = R_3 = Me$; $R_4 = H$
4 $R_2 = R_3 = Me$; $R_4 = Ms$
5 $R_2 = R_3 = Me$; $R_4 = Ac$

6 $R_4 = Me$
7 $R_4 = Ms$
8 $R_4 = Ac$

9 $R_4 = Me$; $R_6 = H$
10 $R_4 = Ms$; $R_6 = H$
11 $R_4 = Ac$; $R_6 = H$
12 $R_4 = Me$; $R_6 = Me$
13 $R_4 = Ms$; $R_6 = Me$
14 $R_4 = Ac$; $R_6 = Me$

15

esters, gave the crystalline unsaturated ester, methyl (methyl 4-deoxy-2,3-di-*O*-methyl-α-L-*threo*-hex-4-enopyranosid)uronate (**15**), in 84–87% isolated yield. The structure of the ester was fully supported by u.v., i.r., n.m.r., and mass spectra. The ease with which β-elimination of methanesulfonic acid takes place with formation of the unsaturated hexuronate (15) when the mesyl ester (**13**) is treated with base was strikingly emphasized during g.l.c. examination of the mesyl ester (**13**). Whereas g.l.c. of the mesyl ester (**13**) on a column coated with neutral silicone gum SE-30 gave a single peak of substantially higher retention time than that of the unsaturated ester (**15**), g.l.c. on a column coated with the nitrile silicone gum XE-60 as liquid phase gave a single peak of the same retention time as the unsaturated ester (**15**). Gas–liquid chromatography – mass spectrometry supported the conclusion that unsaturated ester had been formed on the g.l.c. column.

Bases chosen for the study of their action on the acetylated (**14**) and methylated (**12**) esters were either those of low nucleophilicity towards ester functions which would catalyze elimination with minimum de-esterification or those which would regenerate hexuronic ester functions by base-catalyzed ester exchange although in the latter case de-*O*-acetylation of the 4-*O*-acetate could take place. The results are summarized in Tables 1 and 2.

Treatment of the acetylated ester (**14**) with DBU (1,5-diazabicyclo[5.4.0]undec-5-ene) in benzene at room temperature afforded the crystalline unsaturated ester (**15**) in 83% isolated yield.[3] The unsaturated ester (**15**) was also isolated in 66% yield as the major detectable product when the acetylated ester (**14**) was treated with sodium hydride in ether. The effect of other bases on the acetylated ester was studied only in small-scale experiments in which products were analyzed by g.l.c. and t.l.c. Treatment of the ester (**14**) with methanolic sodium methoxide resulted in both de-*O*-acetylation and β-elimination. This observation has significance for studies on polysaccharides since the reaction conditions, which are those commonly used for the de-*O*-acylation of acylated derivatives, could result in structural modification of hexuronic ester residues. It was also observed that treatment of the acetylated ester (**14**) with acetic anhydride and potassium acetate at 100° caused some β-elimination. A similar observation was made previously by Schmidt and Neukom (11). Although these conditions are harsher than those which need be used for acetylation it is clear that care should be exercized in the acetylation of carbohydrates containing hexuronic ester units lest inadvertent degradation takes place. Alkali metal fluorides may act as strong bases in dipolar aprotic solvents (12), and some β-elimination was observed on prolonged treatment of the acetylated ester (**14**) with cesium fluoride in methyl sulfoxide at room temperature. In contrast, the unusually strong organic base, 1,8-bis (*N*,*N*-dimethylamino)naphthalene (13), was without effect on the acetylated ester (**14**).

The crystalline unsaturated ester (**15**) was isolated in 58% yield on treatment of the methylated ester (**12**) with methylsulfinyl carbanion in methyl sulfoxide. An unknown substance was formed as a minor reaction product and a substance of similar retention time was also detected amongst the products from the action of potassium *t*-butoxide in methyl sulfoxide on the methylated ester (**12**) but in neither

[3]Recently, Llewellyn and Williams (23) have reported a similar reaction of a 4-*O*-acetyl hexuronic ester with DBU.

TABLE 1. Action of bases on methyl (methyl 4-*O*-acetyl-2,3-di-*O*-methyl-β-D-glucopyranosid)uronate (**14**)

Base conditions of reaction[a]	Isolated yield (%) of unsaturated ester (**15**)	Relative proportions of products (by g.l.c.)			
		Acetylated ester (**14**)	Unsaturated ester (**15**)	De-*O*-acetylated ester	Others[b]
DBU/benzene, 5 days, r.t.	85		11.0		1.0 (B)
NaH/ether, 24 h, r.t.	66				
NaOMe/MeOH, 90 min, r.t.			1.3	2.1	1.0 (A)
$KOBu_t$/DMSO, 90 min, r.t.			1.0	1.4	
Ac_2O/KOAc, 7 days, r.t.		Unchanged			
Ac_2O/KOAc, 24 h, 100°		5.0	1.0		
bis(DMA)N/benzene, 10 days, r.t.		Unchanged			
bis(DMA)N/DMF, 8 days, r.t.		Unchanged			
CsF/DMSO, 9 days, r.t.		4.5	1.0		

[a]DBU, 1,5-diazabicyclo[5.4.0]undec-5-ene; DMSO, methyl sulfoxide; bis(DMA)N, 1,8-bis(*N*,*N*-dimethylamino)naphthalene.
[b]A and B are unidentified compounds whose relative retention times are quoted in the Experimental.

TABLE 2. Action of bases on methyl (methyl 2,3,4-tri-*O*-methyl-β-D-glucopyranosid)uronate (**12**)

Base/conditions of reaction[a,c]	Isolated yield (%) of unsaturated ester (**15**)	Relative proportions of products (by g.l.c.)		
		Methylated ester (**12**)	Unsaturated ester (**15**)	Others[b]
NaH/DMSO, 4 h, r.t.	58		11.0	1.0 (C)
NaOMe/MeOH, 4 h, r.t.		10.5	1.9	1.0 (D) 2.4 (E)
NaOMe/MeOH, 4 h, 60°		5.9	3.0	1.0 (D) 2.8 (E)
$KOBu_t$/DMSO, 70 min, r.t.			25.6	1.0 (D*) 2.6 (C*)

[a]See footnote to Table 1.
[b]C, D, and E are unidentified compounds whose relative retention times are quoted in the Experimental section; C* and D* have the same relative retention times as C and D respectively but no other criteria of identity are yet available.
[c]No reaction was observed under the following conditions: (*i*) Ac_2O/NaOAc, 9 days, r.t.; (*ii*) Ac_2O/KOAc, 24 h, 100°; (*iii*) bis(DMA)N/benzene, 10 days r.t.; (*iv*) bis(DMA)N/DMF, 7 days, r.t.; (*v*) DBU/benzene, 9 days, r.t.; (*vi*) DBU/DMF, 7 days, r.t.; (*vii*) CsF/DMSO, 7 days, r.t.; (*viii*) MeI/Ag_2O, 3 days, 35°; (*ix*) MeI/Ag_2O/DMF, 8 days, r.t.; (*x*) NaH/benzene, 4 h, r.t.; (*xi*) NaH/ether, 70 min, r.t.

case was it possible to obtain definite evidence for structure. It was noted, however, that the unsaturated ester (**15**) remained unchanged on treatment with methylsulfinyl carbanion in methyl sulfoxide. Of other bases examined only sodium methoxide in methanol caused formation of the unsaturated ester (**15**) from the methylated ester (**12**). Yoshimura and coworkers (14) have shown that when methyl (methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosid)uronate is treated with sodium methoxide in methanol under a variety of conditions less than 50% conversion into the corresponding unsaturated ester is achieved and that some of the original ester is saponified. In our hands similar treatment of the β-anomer (**12**) with methanolic sodium methoxide in the presence of 2,2-dimethoxypropane to remove traces of water (5) still led to the formation of acidic products. In addition g.l.c. showed that unsaturated ester (**15**) was formed together with two unknown compounds, but under none of the conditions used was methylated ester completely transformed to other products. Treatment of the methylated ester with potassium *t*-butoxide in methyl sulfoxide proceeded to completion with formation of the unsaturated ester as the major product and two unidentified substances as minor products. The absence of detectable β-elimination when the methylated ester (**12**) was treated with silver oxide in methyl iodide alone or with silver oxide and methyl iodide in *N*,*N*-dimethylformamide is significant in view of the widespread use of the Purdie (15) and Kuhn (16) procedures for the methylation of carbohydrates.

The results of these studies on the three 4-*O*-substituted methyl (methyl 2,3-di-*O*-methyl-β-

D-glucopyranosid)uronates clearly indicate that mesylate > acetate > methoxide represents the order of effectiveness of leaving groups in base-catalyzed β-eliminations leading to formation of the unsaturated hexuronate (**15**). A full assessment of the potential degradative applications of β-elimination reactions on interior units within acidic polysaccharide chains cannot be made until the effectiveness of 4-*O*-glycosyl substituents has been examined. Model experiments, presently in progress, will assess both the initial elimination and the effect of bases on the reducing sugar residues which may be exposed as a consequence of such eliminations.

Experimental

Melting points are uncorrected. Optical rotations were measured with a Perkin–Elmer model 141 polarimeter at 20 ± 2°. The i.r. and u.v. spectra were measured on Unicam SP 200 and SP 800A spectrophotometers. The n.m.r. spectra were recorded on a JEOL C-60HL spectrophotometer with tetramethylsilane as internal standard. The t.l.c. was performed with Kieselgel HF 254 as the adsorbent and the dried plates were sprayed with ethanol containing 5% of sulfuric acid and 5% of 1-naphthol and heated at about 150°. Solvent systems used for t.l.c. were chloroform–acetone (20:1 and 4:1) and benzene – light petroleum (b.p. 30–60°) (5:1). Preparative layer separations were performed using ether – light petroleum (b.p. 30–60°) (5:1). Column chromatography was carried out on silica gel (Davison grade 950, 60–200 mesh). Unless otherwise stated light petroleum refers to the fraction of b.p. 30–60°. Solutions were concentrated below 50° under reduced pressure.

Mass spectra were recorded on an A.E.I. MS 12 mass spectrometer, with an ionization potential of 70 eV. The g.l.c. was performed with a Hewlett–Packard model 5750 chromatograph using columns of dichlorodimethylsilane-treated Celite coated with (*a*) 5% of silicone gum XE-60 (operating temperature 150°) and (*b*) 5% of neopentylglycol adipate polyester (operating temperature 200°). For g.l.c. – mass spectrometry column *a* was used in a Pye 104 chromatograph and attached to the mass spectrometer via a Watson–Biemann separator. The mass spectra were recorded at an inlet temperature of 200°, an ionization temperature of 70 eV, and an ion source temperature of *ca.* 150°. Retention times (R_{Me}) of methyl (methyl glycopyranosid)uronates are quoted relative to methyl (methyl 2,3,4-tri-*O*-methyl-β-D-glucopyranosid)uronate (**12**).

Methyl (Methyl 2,3,4-Tri-O-methyl-β-D-glucopyranosid)-uronate (12)

Sodium hydride (22 g) was added with stirring to methyl 6-*O*-trityl-β-D-glucopyranoside (**1**) (17) (17.15 g) in *N*,*N*-dimethylformamide (350 ml) at 0°, and the mixture was stirred for 20 min. Methyl iodide (90 ml) was added dropwise during 15 min to the cooled solution, and the mixture was kept at room temperature for 18 h. The t.l.c. showed that reaction was complete and methanol was added to destroy excess of sodium hydride. Insoluble material was removed by filtration, the filtrate was concentrated to remove methanol and diluted with chloroform, and the chloroform solution was washed with water, dried, and concentrated to give crude methyl 2,3,4-tri-*O*-methyl-6-*O*-trityl-β-D-glucopyranoside (**2**) (14.4 g, 76.6%), $[\alpha]_D$ −95° (*c*, 2.0 in chloroform); n.m.r. data ($CDCl_3$): τ 2.52–2.97 (15-proton multiplet, trityl), 5.91 (1-proton doublet, splitting 7 Hz, H-1), 6.47 (9-proton singlet, 3 OMe), 6.78 (3-proton singlet, OMe).

Hydrogen bromide (40–45%) in acetic acid (6.5 ml) was added with stirring to the foregoing trityl ether (**2**) (14.1 g) in acetic acid (25 ml). After 3 min trityl bromide was removed by filtration, and the filtrate was poured with stirring into ice-cold, saturated, aqueous solid sodium hydrogen carbonate. Solid material was removed by filtration, the filtrate was extracted with chloroform (3 × 250 ml), and the chloroform solution was washed with saturated sodium hydrogen carbonate solution and twice with water, dried and concentrated to give a crystalline residue. Recrystallization from light petroleum (b.p. 40–65°) – ether (19:1) afforded methyl 2,3,4-tri-*O*-methyl-β-D-glucopyranoside (**6**) (5.29 g, 76%), m.p. 92–94°, $[\alpha]_D$ −24.2° (*c*, 2.0 in methanol) (lit. (18); m.p. 93–94°, $[\alpha]_D$ −22.9° (methanol)); ν_{max} (Nujol) 3520 cm^{-1} (OH); n.m.r. data ($CDCl_3$): τ 5.89 (1-proton doublet, splitting 7 Hz, H-1), 6.43 (3-proton singlet, C-1 OMe), 6.48–6.53 (3 × 3-proton singlets, 3 OMe).

Chromium trioxide (4.21 g) in 3.75 *N* sulfuric acid (20 ml) was added dropwise with stirring to methyl 2,3,4-tri-*O*-methyl-β-D-glucopyranoside (**6**) (3.6 g) in acetone (20 ml) at 5°. After 2 h at room temperature the reaction mixture was poured into ethanol (200 ml) containing ice and the supernatant liquid was decanted from the inorganic deposit into water. The aqueous solution was extracted with chloroform (12 × 100 ml) until the aqueous layer gave only a faint Molisch test. The chloroform extracts were washed once with water, dried, and concentrated to a crystalline residue which was recrystallized from ether – light petroleum (b.p. 30–60°) to yield methyl 2,3,4-tri-*O*-methyl-β-D-glucopyranosiduronic acid (**9**) (2.58 g, 77%), m.p. 132–134°, $[\alpha]_D$ −37.1° (*c*, 1.0 in water) (lit. (19); m.p. 133°, $[\alpha]_D$ −38° (water)); ν_{max} (Nujol) 2800 (broad), 1710 cm^{-1} (CO_2H).

Diazomethane (0.055 g) in ether (20 ml) was added with swirling to the foregoing acid (**9**) (0.25 g) in ether (10 ml). The t.l.c. showed complete disappearance of the acid after a few minutes. Concentration of the solution gave a crystalline mass which was recrystallized from light petroleum to give methyl (methyl 2,3,4-tri-*O*-methyl-β-D-glucopyranosid)uronate (**12**) (0.248 g, 94%), m.p. and mixed m.p. 52–53°, $[\alpha]_D$ −36.4°; ν_{max} (Nujol) 1750 cm^{-1} (ester C=O); n.m.r. data ($CDCl_3$): τ 5.88 (1-proton doublet, splitting 7 Hz, H-1), 6.25 (3-proton singlet, ester OMe), 6.45, 6.50, 6.53, 6.57 (4 × 3-proton singlets, 4 OMe). The g.l.c. showed that the ester was anomerically pure. The mass spectra of the ester by direct insertion on the probe and by g.l.c. – mass spectrometry were identical with that reported by Kováčik *et al.* (20) [M^+, 264].

Methyl 2,3-Di-O-methyl-6-O-trityl-β-D-glucopyranoside (3)

Methyl 2,3-di-*O*-methyl-β-D-glucopyranoside (**21**) (12.7 g) and trityl chloride (16.0 g) in pyridine (100 ml) were heated on a boiling-water bath for 1 h and kept at room temperature for 48 h. Water (3 ml) was added to the

solution which was kept for 1 h before being poured into ice-water to give a gummy deposit which was dissolved in chloroform. The chloroform solution was washed with water, dried, and concentrated with repeated addition of toluene in order to remove pyridine to give a syrup (28.2 g) which was shown by t.l.c. to contain the desired trityl ether and tritanol. Chromatography of the syrup on silica gel (1000 g) with light petroleum (b.p. 30–60°)–ether (2:1) as eluant to give methyl 2,3-di-*O*-methyl-6-*O*-trityl-β-D-glucopyranoside (**3**) (24.7 g, 93.2%) as a glassy solid, $[\alpha]_D$ −36.7° (*c*, 2.0 in chloroform) (lit. (22); $[\alpha]_D$ −35° (chloroform)) ν_{max} (Nujol) 3600 cm^{-1} (OH); n.m.r. data ($CDCl_3$): τ 2.52–2.97 (15-proton multiplet, trityl), 5.87 (1-proton doublet, splitting 7 Hz, H-1), 6.47, 6.48 (9 protons, 3 OMe).

*Methyl 4-O-Mesyl-2,3-di-O-methyl-6-O-trityl-β-D-glucopyranoside (**4**)*

The trityl ether (**3**) (9.53 g) was treated with methanesulfonyl chloride (2.0 ml) in pyridine (50 ml) for 14 h at room temperature. The mixture was poured into ice-water, saturated aqueous sodium hydrogen carbonate was added with vigorous stirring, the separated syrup was dissolved in chloroform, and the aqueous solution was extracted with chloroform. The combined chloroform extracts were washed with water, dried, and concentrated to a yellow syrup. Chromatography of the syrup on silica gel with chloroform as eluant gave methyl 4-*O*-mesyl-2,3-di-*O*-methyl-6-*O*-trityl-β-D-glucopyranoside (**4**) (10.4 g, 93%) as a syrup, $[\alpha]_D$ −10.8° (*c*, 2.0 in chloroform) (lit. (22); $[\alpha]_D$ −12° (chloroform)); ν_{max} (film) 1335, 1180 cm^{-1} (S=O); n.m.r. data ($CDCl_3$): τ 2.45–3.00 (15-proton multiplet, trityl), 5.87 (1-proton doublet, splitting 7 Hz, H-1), 6.45 (9-proton singlet, 3 OMe), 7.33 (3-proton singlet, mesyl).

*Methyl 4-O-Mesyl-2,3-di-O-methyl-β-D-glucopyranoside (**7**)*

Hydrogen bromide (40–45%) in acetic acid (4.6 ml) was added to the preceding mesyl ester (**4**) (10.22 g) in acetic acid (25 ml) and after 3 min trityl bromide was removed by filtration and the filtrate was worked-up in the usual way to give a crystalline solid (4.79 g) which was recrystallized from ether – light petroleum (b.p. 30–60°) to furnish methyl 4-*O*-mesyl-2,3-di-*O*-methyl-β-D-glucopyranoside (**7**) (4.40 g, 81.1%), m.p. 84.5–85.5°, $[\alpha]_D$ −29.4° (*c*, 2.0 in chloroform) (lit. (22); m.p. 85.5–86.5°, $[\alpha]_D$ −28° chloroform)); ν_{max} (Nujol) 3530 (OH), 1350, 1185 cm^{-1} (S=O); n.m.r. data ($CDCl_3$): τ 5.85 (1-proton doublet, splitting 7 Hz, H-1), 6.42, 6.46, 6.49 (3 × 3-proton singlets, 3 OMe), 6.89 (3-proton singlet, mesyl).

Anal. Calcd. for $C_{10}H_{20}O_8S$: C, 39.9; H, 6.71; S, 10.68. Found: C, 39.88; H, 6.84; S, 10.52.

*Methyl 4-O-Mesyl-2,3-di-O-methyl-β-D-glucopyranosiduronic acid (**10**)*

Chromium trioxide (1.20 g) in 3.75 *N* sulfuric acid was added dropwise with stirring to the mesyl ester (**7**) (1.20 g) in acetone at 5°, and the mixture was kept at room temperature for 2.5 h. The reaction mixture was neutralized with solid sodium hydrogen carbonate and extracted with chloroform (4 × 75 ml). The aqueous solution was acidified to p*H* 2 and extracted with chloroform (10 × 100 ml) until the aqueous layer gave only a faint Molisch test. The dried chloroform extract was concentrated to a crystalline residue which was recrystallized from ether – light petroleum (b.p. 30–60°) to give methyl 4-*O*-mesyl-2,3-di-*O*-methyl-β-D-glucopyranosiduronic acid (**10**) (1.05 g, 83.3%), m.p. 93–95°, $[\alpha]_D$ +10° (*c*, 2.0 in chloroform); ν_{max} (Nujol) 1740 (acid C=O), 1360, 1180 cm^{-1} (S=O); n.m.r. data ($CDCl_3$): τ 2.25 (1-proton, broad, CO_2H), 5.78 (1-proton doublet, splitting 7 Hz, H-1), 6.44, 6.47, 6.52 (3 × 3-proton singlets, 3 OMe), 6.97 (3-proton singlet, mesyl).

Anal. Calcd. for $C_{10}H_{18}O_9S$: C, 38.11; H, 5.77; S, 10.20. Found: C, 37.91; H, 5.82; S, 10.09.

*Methyl (Methyl 4-O-Mesyl-2,3-di-O-methyl-β-D-glucopyranosid)uronate (**13**)*

Diazomethane (0.15 g) in ether (5 ml) was added with swirling to the mesylated acid (**10**) (0.98 g) in ether (20 ml). The t.l.c. showed that reaction was complete after a few min, and work-up of the solution followed by recrystallization from ethanol gave methyl (methyl 4-*O*-mesyl-2,3-di-*O*-methyl-β-D-glucopyranosid)uronate (**13**) (0.96 g, 94%), m.p. 110–112°, $[\alpha]_D$ −31.6° (*c*, 2.0 in chloroform); ν_{max} (Nujol) 1750 (ester C=O), 1340, 1180 cm^{-1} (S=O); n.m.r. data ($CDCl_3$): τ 5.87 (1-proton doublet, splitting 7 Hz, H-1), 6.27 (3-proton singlet, ester OMe), 6.46, 6.50, 6.54 (3 × 3-proton singlets, 3 OMe), 6.99 (3-proton singlet, mesyl); M^+, 328.

Anal. Calcd. for $C_{11}H_{20}O_9S$: C, 40.24; H, 6.14; S, 9.77. Found: C, 40.11; H, 6.16; S, 9.64.

The g.l.c. on a column coated with silicone gum SE-30 at 152° showed that the ester was homogeneous (R_{Me} 3.16) and g.l.c. – mass spectroscopy, using the same column, gave a mass spectrum identical with that obtained by direct insertion on the probe (found: M^+, 328). The g.l.c. on column *a* gave a single peak of the same retention time (R_{Me} 1.31) as the unsaturated ester, methyl (methyl 4-deoxy-2,3-di-*O*-methyl-α-L-*threo*-hex-4-enopyranosid)uronate(**15**). The g.l.c. – mass spectrometry showed that this component gave a mass spectrum identical with that of the unsaturated ester (**15**) (Found: M^+, 232).

*Methyl 4-O-Acetyl-2,3-di-O-methyl-6-O-trityl-β-D-glucopyranoside (**5**)*

Methyl 2,3-di-*O*-methyl-6-*O*-trityl-β-D-glucopyranoside (**3**) (9.36 g) and acetic anhydride (2.3 ml) in pyridine (50 ml) were kept at room temperature for 14 h, when t.l.c. showed that reaction was complete. The mixture was poured into ice-water containing solid sodium hydrogen carbonate and the gummy deposit which separated was dissolved in chloroform. The chloroform solution was washed with saturated sodium hydrogen carbonate solution and with water, dried, and concentrated to give methyl 4-*O*-acetyl-2,3-di-*O*-methyl-6-*O*-trityl-β-D-glucopyranoside (**5**) (9.53 g, 93.4%), $[\alpha]_D$ −2.1° (*c*, 2.0 in chloroform), as a pale yellow syrup; ν_{max} (film) 1745, 1225 cm^{-1} (acetate); n.m.r. data ($CDCl_3$): τ 2.55–3.00 (15-proton multiplet, trityl), 6.43, 6.47, 6.57 (3 × 3-proton singlets, 3 OMe), 8.24 (3-proton singlet, OAc). The sample prepared for analysis crystallized on standing at 0° and had m.p. 92–94°.

Anal. Calcd. for $C_{30}H_{34}O_7$: C, 71.12; H, 6.76. Found: C, 71.16; H, 6.79.

*Methyl 4-O-Acetyl-2,3-di-O-methyl-β-D-glucopyranoside (**8**)*

Hydrogen bromide (40–45%) in acetic acid (4.3 ml) was

added to the foregoing acetylated trityl ether (**5**) (8.95 g) and after 3 min trityl bromide was removed by filtration and the reaction mixture was worked-up in the usual way to give a crystalline residue (3.90 g). The t.l.c. indicated the presence of a major component and two faster moving minor components. Column chromatography on silica gel with chloroform–ether (2:1) as eluant followed by recrystallization from ether – light petroleum (b.p. 30–60°) afforded methyl 4-*O*-acetyl-2,3-di-*O*-methyl-β-D-glucopyranoside (**8**) (3.48 g, 75%), m.p. 84.0–84.5°, $[\alpha]_D$ −57.1° (*c*, 2.0 in chloroform); ν_{max} (Nujol) 3480 (OH), 1735 cm^{-1} (ester C=O); n.m.r. data ($CDCl_3$): τ 5.84 (1-proton doublet, splitting 7 Hz, H-1), 6.40, 6.46, 6.49 (3 × 3-proton singlets, 3 OMe), 7.91 (3-proton singlet, OAc).

Anal. Calcd. for $C_{11}H_{20}O_7$: C, 50.00; H, 7.63. Found: C, 50.50; H, 7.69.

Methyl 4-O-Acetyl-2,3-di-O-methyl-β-D-glucopyranosiduronic acid (11)

Chromium trioxide (1.80 g) in 3.75 *N* sulfuric acid was added dropwise with stirring to the acetate (**8**) (1.57 g) in acetone (8 ml) at 5° and the mixture was stirred at room temperature for 2 h. The reaction mixture was neutralized with solid sodium hydrogen carbonate and extracted with chloroform. The aqueous solution was acidified to p*H* 2 and extracted with chloroform (10 × 100 ml) until the aqueous layer gave only a faint Molisch test. The chloroform extract was dried and concentrated to a pale yellow syrup which crystallized on standing. Recrystallization from ether – light petroleum (b.p. 30–60°) furnished methyl 4-*O*-acetyl-2,3-di-*O*-methyl-β-D-glucopyranosiduronic acid (**11**) (1.23 g, 75%), m.p. 101–103°, $[\alpha]_D$ −46.8° (*c*, 2.0 in chloroform); ν_{max} (Nujol) 1735, 1235 (acetate), 1700 cm^{-1} (shoulder) (CO_2H); n.m.r. data ($CDCl_3$): τ 0.22 (1-proton singlet, CO_2H), 5.73 (1-proton doublet, splitting 7 Hz, H-1), 6.44, 6.46, 6.49 (3 × 3-proton singlets, 3 OMe), 7.91 (3-proton singlet, OAc).

Anal. Calcd. for $C_{11}H_{18}O_8$: C, 47.28; H, 6.52. Found: C, 47.35; H, 6.51.

Methyl (Methyl 4-O-Acetyl-2,3-di-O-methyl-β-D-glucopyranosid)uronate (14)

Diazomethane (0.105 g) in ether (5 ml) was added with swirling to the foregoing acid (**11**) (0.605 g) in ether (8 ml). The t.l.c. showed that reaction was complete after a few minutes, and work-up of the solution followed by recrystallization from ethanol gave methyl (methyl 4-*O*-acetyl-2,3-di-*O*-methyl-β-D-glucopyranosid)uronate (**14**) (0.588 g, 92.6%), m.p. 97–99°, $[\alpha]_D$ −51.7° (*c*, 2.0 in chloroform); ν_{max} (Nujol) 1750 (broad) (ester C=O), 1240 cm^{-1} (acetate C—O); n.m.r. data ($CDCl_3$): τ 5.85 (1-proton doublet, splitting 7 Hz, H-1), 6.33 (3-proton singlet, ester OMe), 6.47–6.53 (3 × 3-proton singlets, 3 OMe), 7.97 (3-proton singlet, OAc); M^+, 292.

Anal. Calcd. for $C_{12}H_{20}O_8$: C, 49.29; H, 6.90. Found: C, 49.19; H, 6.97.

Methyl (Methyl 4-Deoxy-2,3-di-O-methyl-α-L-threo-hex-4-enopyranosid)uronate (15)

(*a*) Methanolic 1.14 *N* sodium methoxide (0.75 ml) was added with swirling to the mesylated ester (**13**) (0.25 g) in methanol (5 ml). After 5 min sodium methanesulfonate began to precipitate and after 20 min the reaction mixture was only slightly alkaline. Benzene (25 ml) was added and the solution was filtered and concentrated to a syrup which crystallized on standing. Recrystallization from cyclohexane furnished methyl (methyl 4-deoxy-2,3-di-*O*-methyl-α-L-*threo*-hex-4-enopyranosid)uronate (**15**) (0.154 g, 87%), which was homogeneous on g.l.c. and t.l.c. and had m.p. 51–53°, $[\alpha]_D$ +48° (*c*, 2.0 in methanol); ν_{max} (Nujol) 1735 (ester C=O), 1635 cm^{-1} (C=C); λ_{max} (MeOH) 236 nm (ε 6200); n.m.r. data ($CDCl_3$): τ 3.83 (1-proton doublet, splitting 4 Hz, H-4), 5.07 (1-proton doublet, splitting 4 Hz, H-1), 6.26 (3-proton singlet, ester methoxyl), 6.50, 6.53, 6.60 (3 × 3-proton singlets, 3 OMe); M^+, 232; g.l.c. – mass spectrometry of the ester gave a spectrum (M^+, 232) identical to that obtained by direct insertion on the probe.

Anal. Calcd. for $C_{10}H_{16}O_6$: C, 51.72; H, 6.94. Found: C, 51.66; H, 6.99.

(*b*) Ethanolic 0.5 *N* potassium hydroxide (1.1 ml) was added with swirling to the mesylated ester (**13**) (0.156 g) in ethanol (5 ml). After 10 min potassium methanesulfonate had precipitated and after 30 min the reaction mixture was only slightly alkaline. Benzene (20 ml) was added and the solution was filtered, concentrated, and recrystallized from cyclohexane to give the unsaturated ester (**15**) (0.092 g, 83.6%), m.p. and mixed m.p. 51–52°, $[\alpha]_D$ +48.5° (*c*, 2.0 in methanol); ν_{max} (Nujol) 1735 (ester C=O), 1635 cm^{-1} (C=C); λ_{max} 236 nm (ε 6170); M^+, 232.

(*c*) Sodium hydride (11 mg) in ether (5 ml) was added to the acetylated ester (**14**) (0.085 g) in ether (10 ml) and the mixture was stirred vigorously at room temperature for 70 min. A calculated quantity (1.2 *M* excess with respect to hydride) of benzoic acid (1.1 mg/ml) in ether was added to destroy excess of hydride. The solution was shaken with saturated aqueous sodium hydrogen carbonate, dried, and concentrated to a syrup. The t.l.c. showed the presence of a major component with the mobility of the unsaturated ester (**15**), traces of starting material (**13**), and a minor component of lower mobility. The g.l.c. on column *a* showed that the major component had the retention time (R_{Me} 1.32) of the unsaturated ester (**15**) and that a trace of starting material (R_{Me} 4.15) was present while g.l.c. – mass spectrometry of the major component gave a mass spectrum identical with that of the unsaturated ester (**15**). The syrup was separated by preparative layer chromatography to give the unsaturated ester (**15**) (0.044 g, 66%), m.p. and mixed m.p. 51–53°, whose i.r. and n.m.r. spectra were identical with those of previously prepared samples.

(*d*) 1,5-Diazabicyclo[5.4.0]undec-5-ene (0.020 g) was added to the acetylated ester (**14**) (0.130 g) in benzene (2.5 ml), and the solution was kept at room temperature for 5 days when t.l.c. indicated disappearance of starting material and g.l.c. on column *a* showed the presence of unsaturated ester (R_{Me} 1.35) as the major component together with an unknown substance (R_{Me} 1.78). The solution was neutralized by the addition of methanolic hydrogen chloride, diluted with chloroform, washed with water, dried, and concentrated to a syrup (0.094 g). The syrup was separated by preparative layer chromatography and the major fraction was recrystallized from cyclohexane to give the unsaturated ester (**15**) (0.085 g, 83%), m.p. and mixed m.p. 50–52°, which was homogeneous on t.l.c. and g.l.c.

(*e*) Sodium hydride (0.026 g) was dissolved in methyl sulfoxide (10 ml) and the solution of the methylsulfinyl carbanion was added slowly with stirring to the methylated

ester (**12**) (0.238 g) in methyl sulfoxide (15 ml) and the reaction mixture was kept at room temperature for 4 h when t.l.c. showed complete disappearance of starting material and g.l.c. on column *a* indicated the formation of unsaturated ester (R_{Me} 1.35) and an unknown substance C (R_{Me} 1.54). The reaction mixture was neutralized by the addition of a slight excess of 0.033 *N* hydrochloric acid, excess of acid was destroyed with sodium hydrogen carbonate, and the solution was extracted twice with chloroform. The dried chloroform extract was concentrated to a syrup which was separated by preparative layer chromatography to give a semi-crystalline material which was recrystallized from cyclohexane to give the unsaturated ester (**15**) (0.122 g, 58%), m.p. and mixed m.p. 51–53°, $[\alpha]_D$ +47° (*c*, 2.0 in methanol); ν_{max} (Nujol) 1740 (ester C=O), 1650 cm^{-1} (C=C); λ_{max} 236 nm (ε 6140); whose n.m.r. and mass spectra were identical to those of previously prepared samples.

A sample of the unsaturated ester (**15**) was treated with methylsulfinyl carbanion in methyl sulfoxide under the above conditions. The g.l.c. showed unchanged starting compound and no other detectable product.

*Action of Other Bases on Methyl (Methyl 4-O-Acetyl-2,3-di-O-methyl-β-D-glucopyranosid)uronate (**14**)*

Methanolic 1.42 *N* sodium methoxide (0.30 ml) containing 5% of 2,2-dimethoxypropane was added to acetylated ester (**14**) (0.017 g) in methanol (1 ml) containing 5% of 2,2-dimethoxypropane and the reaction mixture was kept at room temperature. Aliquot portions were withdrawn, neutralized with methanolic hydrogen chloride, and diluted with chloroform, and the chloroform solution was washed with water, dried, concentrated, and examined by g.l.c. and t.l.c. After 90 min starting material had virtually disappeared and g.l.c. on column *a* showed the formation of unsaturated ester (**15**) (R_{Me} 1.33) and an unknown substance A (R_{Me} 2.55) in the relative proportions of 1.3:1. The t.l.c. showed the presence of an immobile component corresponding to acidic products in addition to two mobile components. Since the g.l.c. columns failed to reveal even standard samples of methyl (methyl 2,3-di-*O*-methyl-β-D-glucopyranosid)uronate, the presence of this compound in the reaction mixture was demonstrated by re-acetylation with acetic anhydride in pyridine. The g.l.c. examination of this product on column *a* showed the presence of unsaturated ester (**15**), unknown substance A, and acetylated ester (R_{Me} 4.17) in the relative proportions of 1.3:1:4.1.

Potassium *t*-butoxide (0.012 g) in methyl sulfoxide (0.6 ml) was added with stirring to acetylated ester (**14**) (0.018 g) in methyl sulfoxide (1 ml). The reaction products were examined in the same general way as above and after 90 min starting material had virtually disappeared. Re-acetylation of the reaction product followed g.l.c. on column *b* showed the presence of unsaturated ester (**15**) (R_{Me} 1.10) and acetylated ester (**14**) (R_{Me} 3.70) in the relative proportions of 1:1:4.

The acetylated ester (**14**) was treated with other bases under the conditions summarized in Table 1 and reactions were assessed by g.l.c. and t.l.c.

*Action of Other Bases on Methyl (Methyl 2,3,4-Tri-O-methyl-β-D-glucopyranosid)uronate (**12**)*

Methanolic 1.14 *N* sodium methoxide (0.18 ml) containing 5% of 2,2-dimethoxypropane was added with stirring to methylated ester (**12**) (0.018 g) in methanol (2 ml) containing 5% of 2,2-dimethoxypropane. The reaction mixture was worked-up after 90 min at room temperature and the u.v. spectrum showed a broad band at 236 nm. The g.l.c. examination of the reaction product on column *a* showed the presence of methylated ester (**12**), unsaturated ester (**15**) (R_{Me} 1.32), and unknown substances D (R_{Me} 0.87) and E (R_{Me} 1.22) in the relative proportions of 10.5:1.9:1.0:2.9. When examined by g.l.c. – mass spectrometry the peak of R_{Me} 1.32 gave a mass spectrum identical to that of the unsaturated ester (**15**). The t.l.c. showed a similar mixture of products together with an immobile component characteristic of uronic acids. The reaction was repeated at 60° for 90 min and g.l.c. of the products showed the presence of the same volatile components in the relative proportions of 5.9:2.8:1.0:2.8.

Potassium *t*-butoxide (0.010 g) in methyl sulfoxide (2 ml) was added with stirring under a stream of nitrogen to methylated ester (**12**) (0.015 g) in methyl sulfoxide (2 ml). Starting material had disappeared after 70 min at room temperature and g.l.c. of the reaction product on column *a* showed the presence of unsaturated ester (**15**) and unknown compounds C* (R_{Me} 0.86) and D* (R_{Me} 1.55) in the relative proportions of 25.6:1.0:2.6.

The methylated ester (**12**) was treated with other bases under the conditions summarized in Table 2 and reactions were assessed by g.l.c. and t.l.c.

The authors wish to acknowledge the award of a Province of Ontario Graduate Fellowship (to P.E.B.), a grant from The Institute of Paper Chemistry, Appleton, Wisconsin, under the Pioneering Research Program, and financial support from the National Research Council of Canada.

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