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β-ELIMINATION OF 2-*O*-(4-*O*-METHYL-α-D-GLUCOPYRANOSYLURONIC ACID)-D-XYLOSE WITH METHYLSULPHINYL CARBANION AND HYDROLYSIS OF THE HEX-4-ENOPYRANOSIDURONIC LINKAGE

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ABSTRACT

On treatment with M sodium methylsulphinylmethanide at 25°, 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose (**1**) was rapidly degraded by β-elimination, to form 2-*O*-(4-deoxy-β-L-*threo*-hex-4-enopyranosyluronic acid)-D-xylose (**2**). The kinetics of hydrolysis of **1** and **2** in 0.5M sulphuric acid have been studied. Compound **2** was hydrolysed 70 times faster than **1**. Compared with the rate coefficients of other related compounds[1,2], **2** was hydrolysed at approximately the same rate as 2-*O*-(4-*O*-methyl-α-D-glucopyranosyl)-D-xylose, 3.5 times more slowly than xylobiose, and twice as fast as the xylosidic bond in *O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-(1→2)-*O*-β-D-xylopyranosyl-(1→4)-D-xylose.

INTRODUCTION

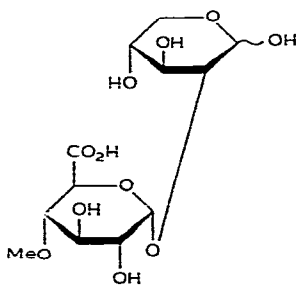
Acidic polysaccharides and aldobiouronic acids containing 4-*O*-substituted hexuronic acid residues undergo[3–5] degradation by β-elimination during methylation by the Hakomori method[6], to give permethylated hex-4-enopyranosiduronate derivatives to appreciable extents.

The Hakomori methylation-method involves two steps: in the first (ionisation) step, the sample is exposed to the strong base sodium methylsulphinylmethanide in methyl sulphoxide for long periods, depending on its solubility; in the second step, methyl iodide is added and reacts readily with the anions formed[7]. It is assumed that the uronic acid residues originally carrying substituents at C-4 undergo β-elimination in the ionisation step.

Hex-4-enopyranosyluronic acid residues have been considered to be extremely acid labile[8], but it was observed that a hex-4-enopyranosyluronic linkage survived[3,4] hydrolysis with 2M trifluoroacetic acid at 100° for 2 h and methanolysis with boiling, anhydrous 1% methanolic hydrogen chloride for 2 h. More exact information on these points is now reported.

RESULTS AND DISCUSSION

2-*O*-(4-*O*-Methyl-α-D-glucopyranosyluronic acid)-D-xylose (**1**) was treated with M sodium methylsulphinylmethanide in methyl sulphoxide under nitrogen at 25° for various periods of time, and the mixtures were then neutralised and subjected to ion-exchange chromatography. The product mixture contained mainly **1**, 2-*O*-(4-deoxy-β-L-*threo*-hex-4-enopyranosyluronic acid)-D-xylose (**2**), and D-xylose (see Fig. 1).



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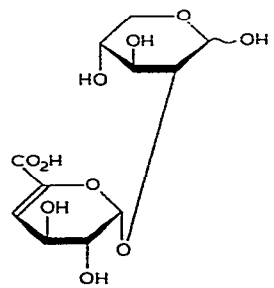

2

Olefin **2** had λ_{max} 230 nm and reacted with thiobarbituric acid to yield a pink product (λ_{max} 550 nm), which are characteristics of a hex-4-enopyranosyluronic acid residue[9,10]. The ^{13}C-n.m.r. spectrum of **2** showed signals at 112.7 and 148.1 p.p.m.

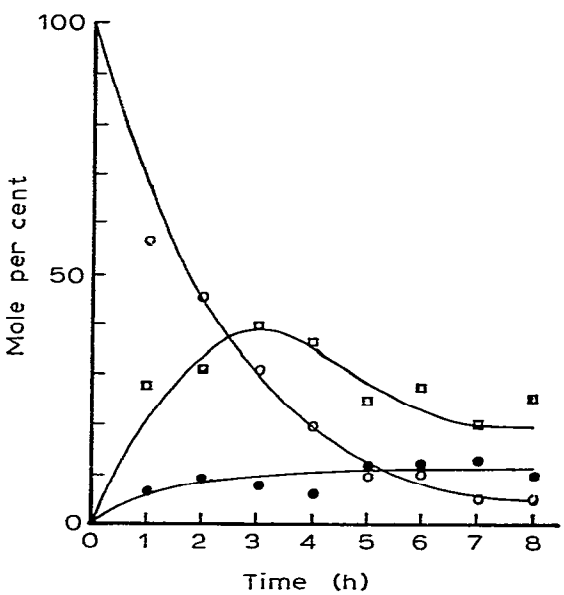

Fig. 1. Composition of the reaction mixture: 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose (**1**) (—○—); 2-*O*-(4-deoxy-β-L-*threo*-hex-4-enopyranosyluronic acid)-D-xylose (**2**) (—□—); and D-xylose (—●—).

assignable to C-4′ and C-5′, respectively, of a hex-4-enopyranosyluronic acid residue[11]. Olefin **2** gave D-xylose on hydrolysis and, on methylation, permethylated derivatives that showed the same retention times in g.l.c. and mass spectra as authentic samples[5].

Fig. 1 shows that **1** was rapidly degraded by β-elimination with sodium methylsulphinylmethanide to form **2**. The concentration of **2** reached a maximum after 3 h, and then decreased. The concentration of D-xylose liberated was slight and levelled off after 2 h, indicating that D-xylose was further degraded in the basic medium and that the degradation of **1** also took place from the reducing xylose residue.

It may be concluded here that 4-*O*-substituted uronic acid residues undergo β-elimination in the ionisation step. It is well known that 4-*O*-substituted, esterified hexuronic acids are easily degraded by β-elimination with bases of low nucleophilicity towards the ester function, so that the driving force of the alkoxycarbonyl group is maintained. Recently, Johansson and Samuelson[12] reported that treatment of 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylitol with M sodium hydroxide at 150° gave a β-elimination product. These results indicate that 4-*O*-substituted hexuronic acid residues undergo degradation by β-elimination in basic media, although the

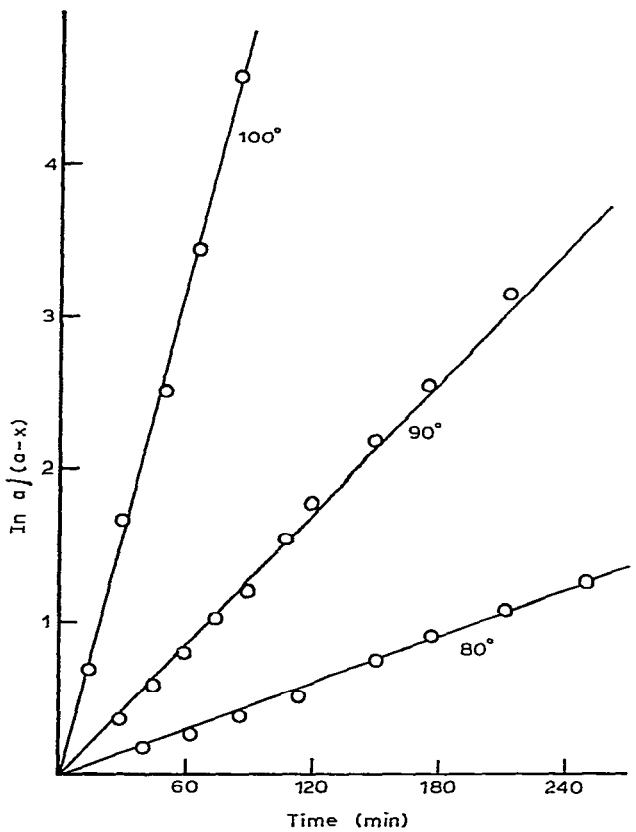

Fig. 2. Rate of hydrolysis of 2-*O*-(4-deoxy-β-L-*threo*-hex-4-enopyranosyluronic acid)-D-xylose (**2**) in H_2SO_4: a, initial conc. of **2**; x, conc. of unhydrolysed **2** after hydrolysis.

TABLE I

KINETIC DATA FOR THE HYDROLYSIS OF COMPOUNDS **1** AND **2**

Compound	k × 10^6 *(sec^{-1})*			E	S
	80°	*90°*	*100°*	*(kcal.mol^{-1})*	*(cal.deg^{-1}.mol^{-1})*
2	99	255	863	28.5	+4.0
1	1.2	4.4	12.7	31.3	+3.5
1[a]	1.22	4.6	14.2	32.4	+3.5
2-*O*-(4-*O*-Me-α-D-Glc*p*)-D-Xyl[a]	59.5	234	746	33.0	+16.1
Xylobiose[a]	274	1018	3089	32.7	+18.3
Xylosidic bond in *O*-(4-*O*-Me-α-D-GlcA*p*)-(1→2)-*O*-β-D-Xyl*p*-(1→4)-D-Xyl	43.4	162	505	32.4	+13.8

[a]Reported by Roy and Timell[1,2], and re-calculated to be valid at 90° and 100° with the aid of the respective energy of activation.

carboxylate anion has much less electron-withdrawing capacity than alkoxycarbonyl groups.

It is generally accepted that hex-4-enopyranosiduronic acid residues are extremely acid labile[8]. However, 2-*O*-(4-deoxy-2,3-di-*O*-methyl-β-L-*threo*-hex-4-enopyranosyluronic acid)-3-*O*-methyl-D-xylose was isolated[3,4] as one of the products after Hakomori methylation and subsequent hydrolysis with 2M trifluoroacetic acid of birch 4-*O*-methylglucuronoxylan, indicating that the hex-4-enopyranosiduronic linkage is stable under conditions that hydrolyse the xylosidic linkages in the xylan main-chain. In order to verify this fact, the rates of hydrolysis of **1** and **2** in 0.5M sulphuric acid at 80–100° were studied. The rates of hydrolysis of **2** are presented in Fig. 2. A straight line was obtained for each set of reaction conditions. The first-order reaction rate-coefficients calculated from the data are listed in Table I. It is well known that compound **1** is quite resistant to acid hydrolysis. The kinetic data reported by Roy and Timell[1,2] are also included in Table I, and the published rate-coefficients have been re-calculated to be valid at 90° and 100° with the aid of the respective energy of activation. The kinetic data for **1** were in good agreement with those reported by Roy and Timell.

Compound **2** was hydrolysed 70 times faster than **1**, indicating that the double bond at C-4 and C-5 decreased considerably the stability to acid hydrolysis of the α-(1→2) inter-sugar linkage, whereas **2** was hydrolysed at approximately the same rate as 2-*O*-(4-*O*-methyl-α-D-glucopyranosyl)-D-xylose, 3.5 times more slowly than xylobiose, and twice as fast as the xylosidic bond in *O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-(1→2)-*O*-β-D-xylopyranosyl-(1→4)-D-xylose.

The activation energy of **2** was 3–4 kcal.mol^{-1} lower than those of other compounds listed in Table I, and the entropy of activation was in the range for **1** and considerably lower than for the neutral glycosides. These facts suggest that **2** was hydrolysed by the same mechanism as **1**, but in a manner different from that of the

neutral glycoside. To discuss the reaction mechanism of hydrolysis, information on the hydrolysis products is vital, but only xylose was detected in the hydrolysate. As the hydrolysis proceeded, the solution became brown. Lindberg *et al.*[8] suggested the formation of a furan derivative on hydrolysis of hex-4-enopyranosiduronic acid residues. This furan derivative might be unstable under the conditions used and be subject to further degradation and/or polymerisation.

EXPERIMENTAL

General methods. — G.l.c., g.l.c.–m.s., and other methods were performed as previously described[5].

Ion-exchange chromatography. — Acidic sugars were separated by chromatography[13] on Aminex A-27 (AcO^-) resin by elution with M acetic acid. The eluate was monitored by differential refractometry for isolation purposes. For quantitative analysis, the eluate was analysed by the orcinol method[14]. The volume distribution coefficients (D_v) were calculated[15] from the peak elution-volumes. Neutral sugar was analysed by a Technicon sugar analyser following the procedure of Sinner *et al.*[16].

Treatment of 2-O-(*4-O-methyl-α-*D-*glucopyranosyluronic acid*)-D-*xylose* (**1**) *with methylsulphinyl carbanion.* — Compound **1** (5 mg, free acid and/or its sodium salt)[13] was mixed with M sodium methylsulphinylmethanide (1 ml) in methyl sulphoxide (1 ml) in a capped serum-bottle under nitrogen. The solution was agitated for various periods of time in an ultrasonic bath (maintained at 25 ±0.01° by a Haake Type FE constant-temperature circulator). Then, 50% aqueous acetic acid was added with external cooling until pH 6 was reached. The resulting solution was applied to a column of Dowex 1-X8 (AcO^-) resin, which was first eluted with water and then with 30% acetic acid.

The aqueous eluate was treated with Dowex 50W (H^+) resin, to remove sodium ions, and then evaporated to dryness under diminished pressure (boiling temperature, ~80° for methyl sulphoxide). The residue was analysed by a Technicon sugar analyser[16], indicating the presence of D-xylose.

The acidic fraction (eluted with 30% acetic acid) was chromatographed on Aminex A-27 (AcO^-) resin by elution with M acetic acid, to give olefin **2** (D_v 15.30) and unreacted **1** (D_v 4.52) together with a small proportion of unknown substances which were not further examined.

Olefin **2** afforded D-xylose on hydrolysis with 2M trifluoroacetic acid at 120° for 2 h, and had $[\alpha]_D^{25}$ +102° (*c* 0.52, water), $\lambda_{max}^{H_2O}$ 230 nm (ε 3900). Reaction of **2** with thiobarbituric acid yielded a pink product (λ_{max} 550 nm)[9]. ^{13}C-N.m.r. data: δ 166.9 (C-6′), 148.1 (C-5′), 112.7 (C-4′), 99.6 (C-1′, MeO-1α), 98.0 (C-1′, MeO-1β), 97.4 (C-1β), 90.7 (C-1α), 80.6 (C-2β), 78.2 (C-2α), 75.0 (C-3β), 72.0 (C-3α), 70.8 (C-4α and β), 70.0 (C-2′), 66.6 (C-3′), 66.0 (C-5β), and 61.7 (C-5α).

Anal. Calc. for $C_{11}H_{16}O_{10}$: C, 42.86; H, 5.23; equiv. wt., 308. Found: C, 42.43; H, 5.24; equiv. wt., 320.

On methylation by Hakomori's method[6,17], compound **2** gave methyl 3,4-di-

O-methyl-2-*O*-(methyl 4-deoxy-2,3-di-*O*-methyl-β-L-*threo*-hex-4-enopyranosylurona-te)-α- and -β-D-xylopyranoside, which showed relative retention-times in g.l.c. and mass spectra identical with those of authentic samples[5].

Acid hydrolysis of compounds **1** *and* **2**. — Compounds **1** and **2** (1.5 mol × 10^{-5}) were hydrolysed with 0.5M H_2SO_4 (10 ml) at 80–100° in stoppered tubes immersed in a thermostated bath, the temperature of which was kept constant within ±0.01°. Samples (100 μl) were withdrawn at appropriate intervals, and the progress of the hydrolysis was followed by determining unchanged starting-material by ion-exchange chromatography. First-order rate coefficients were calculated from the integrated, first-order rate equation.

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