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FORMATION OF ANHYDRIDES OF 2-*O*-(4-*O*-METHYL-α-D-GLUCOPYRANOSYLURONIC ACID)-D-XYLOSE

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ABSTRACT

Three previously unknown acids present in hydrolyzates of 4-*O*-methylglucuronoxylan have been isolated and identified as 4-*O*-methyl-α-D-glucopyranuronic acid α-D-xylopyranose 1,2′:2,1′-dianhydride, 4-*O*-methyl-α-D-glucopyranuronic acid β-D-xylopyranose 1,2′:2,1′-dianhydride, and 4-*O*-methyl-α-D-glucopyranuronic acid α-D-xylofuranose 1,2′:2,1′-dianhydride. In acid media, an equilibrium exists between these dianhydrides and 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose.

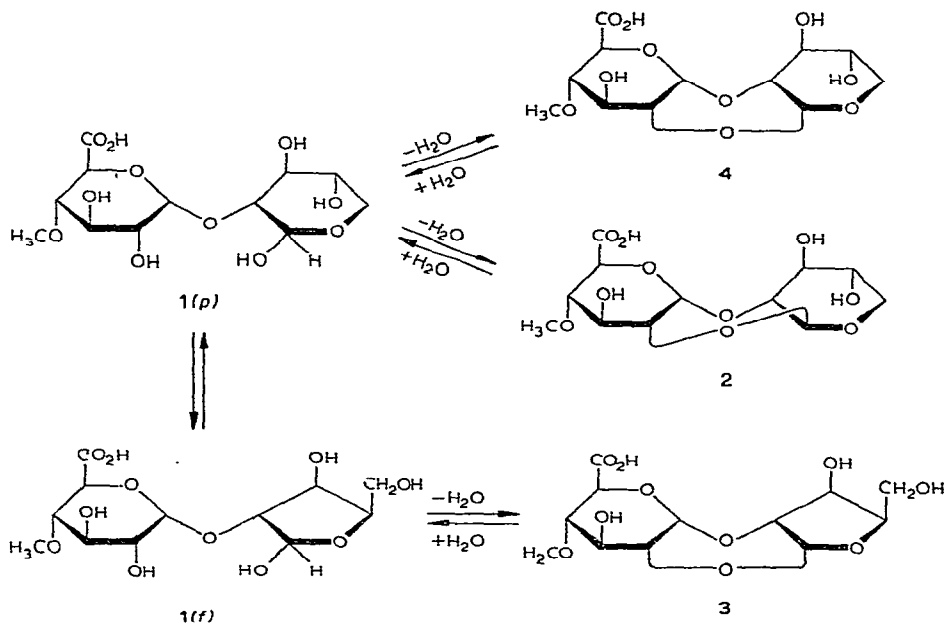
INTRODUCTION

Hydrolysates of polysaccharides containing 4-*O*-methylglucuronoxylan contain appreciable amounts of three unknown monocarboxylic acids, which can be separated by anion-exchange chromatography and which give a strongly positive reaction with carbazole[1]. In the present work, these acids were isolated (see Experimental), and identified, on the evidence cited below, as dianhydrides (**2–4**) of 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose (**1**).

RESULTS AND DISCUSSION

Hydrolysis of **2–4** (0.05M sulphuric acid, 130°, 3 h) gave xylose and 4-*O*-methylglucuronic acid in about equal amounts, together with an appreciable quantity of **1**. For **2** and **4**, a substantial proportion of the starting material remained and small proportions of the other two dianhydrides were formed. Compound **3** behaved similarly except that only a small proportion of the starting material remained. Hydrolysis of **1** gave the above hydrolysis products and small proportions of **2–4**. Trace amounts of lyxose and 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-lyxose were formed in all the hydrolyses[2].

Thus, **1–4** are interconvertible in acid media, **3** has the lowest stability, and **2–4** are anhydrides of **1**. A similar interconversion has been observed between α-D-galactopyranuronic acid β-L-rhamnopyranose 1,2′:2,1′-dianhydride and 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose[3].



Compounds **2**–**4** were not reduced by borohydride[4], demonstrating that C-1 of the xylose moieties is involved in glycosidic bonds. G.l.c.–m.s. of the trimethylsilyl (Me_3Si) derivatives[5] of **2**–**4** revealed peaks at *m/e* 595, which are consistent with the anticipated M − 15 ions and confirm the conclusion that **2**–**4** are dianhydrides.

In order to establish the position of the second glycosidic linkage, **2**–**4** were subjected in sequence to methylation (Hakomori[6]), reduction with lithium aluminium hydride, and hydrolysis (0.5M sulphuric acid, 100°, 4 h). G.l.c.–m.s. of the Me_3Si derivatives[5] of the products revealed that **4** gave mainly 3,4-di-*O*-methylpentopyranose[7]. Two other prominent products were identified as 3,4-di-*O*-methylhexopyranoses[8]. Thus, **4** contains a xylopyranose moiety, and a glycosidic bond between xylose and HO-2 in 4-*O*-methylglucuronic acid, in addition to that between 4-*O*-methylglucuronic acid and HO-2 in xylose. Therefore, **4** is a 4-*O*-methyl-α-D-glucopyranuronic acid D-xylopyranose 1,2′:2,1′-dianhydride.

Compound **2** gave only a 3,4-di-*O*-methylpentopyranose, so that the 4-*O*-methylglucuronic acid moiety must have been destroyed. Thus, **2** contains a xylopyranose moiety with HO-2 blocked by a glycosidic bond and must therefore be a 4-*O*-methyl-α-D-glucopyranuronic acid D-xylopyranose 1,2′:2,1′-dianhydride, since for steric reasons only HO-2 in the 4-*O*-methylglucuronic acid moiety can be involved in a glycosidic bond with the xylopyranose moiety.

Compound **3** gave three products, two of which were identified as 3,4-di-*O*-methylhexopyranoses. The mass spectrum of the third compound strongly indicated a 3,5-di-*O*-methylpentofuranose derivative. Thus, **3** contains a xylofuranose moiety, and glycosidic bonds between xylose and HO-2 in 4-*O*-methylglucuronic acid and

between 4-*O*-methylglucuronic acid and HO-2 in xylose, and therefore is a 4-*O*-methyl-α-D-glucopyranuronic acid D-xylofuranose 1,2′:2,1′-dianhydride. The more rapid hydrolysis of **3** in comparison with **2** and **4** is also consistent with the presence of a xylofuranose moiety.

As expected, compounds **2** and **4**, but not **3**, were oxidized by periodate. Thus, **3** contains no vicinal hydroxyl groups, and the result confirms the presence of a xylofuranose moiety.

The formation of only one biouronic acid (**1**) on hydrolysis of **2–4** is due to the stabilizing effect of the carboxyl group on the glycosidic bond of biouronic acids. Pseudobiouronic acids lack this stabilizing effect and are, if formed, easily hydrolysed[9].

In the formation of **2–4**, the original α-glycosidic bond of the biouronic acid must be unchanged. For **3**, $[\alpha]^{25}$ +68° (*c* 1, water), which contains a xylofuranose moiety, HO-1 and HO-2 must be *cis* in the α form and *trans* in the β form. The latter structure involves great strain, and this explains why only the α form was detected.

For **2**, $[\alpha]_D^{25}$ +91° (*c* 1, water), and **4**, $[\alpha]_D^{25}$ +114° (*c* 1, water), which contain xylopyranose moieties, their $[\alpha]_D$ values suggest that **4** is the α form and **2** the β form.

The D_v values[10] (Table I) obtained for anion-exchange chromatography in acetic acid, sodium acetate, and potassium tetraborate show that the best separations of **2–4** were obtained in acetic acid. In this medium, D_v values increase with increased acid strength[11]. Since the dianhydrides **2–4** are held much more strongly than the biouronic acid **1**, the second glycosidic bond causes a large increase in acid strength. Reduction of the biouronic acid with borohydride has only a slight influence on the acid strength.

TABLE I

VOLUME DISTRIBUTION COEFFICIENTS (D_v)

Acids	*AcOH* (M)	*AcOH* (*0.5*M)	*NaOAc*[a] (*0.08*M)	$K_2B_4O_7$ (*0.15*M)	B *value*
4-*O*-Me-α-D-Glc*p*A β-D-Xyl*p* 1,2′:2,1′-dianhydride (**2**)	11.1		6.38	2.14	0.34
4-*O*-Me-α-D-Glc*p*A α-D-Xyl*f* 1,2′:2,1′-dianhydride (**3**)	20.0		6.87	4.23	0.62
4-*O*-Me-α-D-Glc*p*A α-D-Xyl*p* 1,2′:2,1′-dianhydride (**4**)	23.5		7.06	2.68	0.38
O-(4-*O*-Me-α-D-Glc*p*A)-(1→2)-D-xylose (**1**)	5.6	12.4	3.34	2.64	0.79
O-(4-*O*-Me-α-D-Glc*p*A)-(1→2)-D-xylitol		10.9	2.20	2.83	1.29

[a]Adjusted to pH 5.9 with acetic acid.

D_v values also decrease with increase in the number of hydroxyl groups[11]. This factor has a dominant influence upon the elution order in sodium acetate, and the D_v values of the dianhydrides were much higher than those of the biouronic acid.

In borate medium, the formation of borate complexes leads to an increased charge of the anions. The ratio of D_v in borate medium to that in sodium acetate (*B* value) indicates complexing ability[12]. The *B* values of **2** and **4** reflect a low ability to form complexes. The higher *B* value of **3** is probably due to complex formation

with the xylofuranose moiety. The difference in *B* values between **1** and its anhydrides can be explained by the enhanced ability for complex formation in the furanoid forms (*cf.* **3**). The high *B* value of the borohydride-reduced acid, in comparison to those of the other acids, can be ascribed to the ability of the alditol to form strong borate complexes. The xylitol moiety linked at position 2 can form a 3,4- or a 4,5-complex.

The relative retention times in g.l.c. of the Me_3Si derivatives of the dianhydrides and related compounds are given in Table II. Compound **4** is well separated from **2** and **3** on all stationary phases, and the same holds true for the products formed after reduction with $LiAlH_4$. The relative retention on moderately polar (OV-17, QF-1) and nonpolar (OV-1) stationary phases indicates that the ring with the two glycosidic bonds gives rise to interactions with polar groups in the stationary phases.

TABLE II

G.L.C. DATA[a] FOR DIANHYDRIDES AND RELATED DISACCHARIDES AS TRIMETHYLSILYL DERIVATIVES

	OV-1 240°	*OV-17* 240°	*QF-1* 200°
4-*O*-Me-α-D-Glc*p*A β-D-Xyl*p* 1,2′:2,1′-dianhydride (**2**)	0.382	1.043	0.978
4-*O*-Me-α-D-Glc*p*A α-D-Xyl*f* 1,2′:2,1′-dianhydride (**3**)	0.369	1.073	1.003
4-*O*-Me-α-D-Glc*p*A α-D-Xyl*p* 1,2′:2,1′-dianhydride (**4**)	0.507	1.406	1.439
4-*O*-Me-α-D-Glc*p* β-D-Xyl*p* 1,2′:2,1′-dianhydride	0.271	0.631	0.432
4-*O*-Me-α-D-Glc*p* α-D-Xyl*f* 1,2′:2,1′-dianhydride	0.289	0.696	0.440
4-*O*-Me-α-D-Glc*p* α-D-Xyl*p* 1,2′:2,1′-dianhydride	0.401	0.924	0.779
O-(4-*O*-Me-α-D-Glc*p*A)-(1→2)-D-xylose (**1**)	0.492	0.913	0.814
	0.576	1.126	1.003
O-(4-*O*-Me-α-D-Glc*p*A)-(1→2)-D-xylitol	0.526	0.808	0.736
O-(4-*O*-Me-α-D-Glc*p*)-(1→2)-D-xylitol	0.414	0.531	0.385

[a]Retention times relative to those of the *O*-β-D-glucopyranosyl-(1→4)-D-glucitol derivative.

The formation of **2**–**4** must be considered when determining the total amount of 4-*O*-methylglucuronic acid and xylose in appropriate polysaccharides. Moreover, it is likely that **2** and **4** are present as non-reducing xylan end-groups in pulps which have been treated in acid media. An equilibrium exists between 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose and an anhydride[3]. This anhydride has been isolated after acid hydrolysis of karaya gum[3] and is present in sulphite spent-liquor[13]. Thus, an equilibrium probably exists between (1→2)-linked biouronic acids and their anhydrides in acid media, *e.g.*, in hydrolysates containing the original biouronic acid.

Dianhydrides of diketoses have been isolated after acid treatment of the monosaccharides or their (2→1)-linked polysaccharides[14]. This allows the conclusion that monosaccharides with hydroxyl groups adjacent to their reducing carbonyl group can take part in the formation of dianhydrides if the steric hindrance is not too great.

EXPERIMENTAL

The g.l.c. columns used were those previously described[5]. Mass spectra were recorded with an AEI MS-20 instrument connected *via* a Biemann separator to a Varian model 1200 gas chromatograph.

Isolation of dianhydrides. — Birch meal (50 g) was extracted with acetone and then allowed to swell for 4 days in trifluoroacetic acid (1 litre) at room temperature. Water (100 ml) was then slowly added with stirring and the temperature was raised to 100°. After 30 min, more water (100 ml) was added, followed by 4 similar amounts at intervals of 30 min, and the hydrolysis at 100° was continued for a total of 2.5 h. The solution was then diluted to twice its volume with water, and concentrated at 30° to a small volume. The procedure was repeated five times to remove trifluoroacetic acid. Undissolved material (lignin) was removed, sugars and acids in the filtrate were separated[15], and the acid fraction was subjected to anion-exchange chromatography in 0.08M sodium acetate. Since the individual compounds are not well-separated in this medium (Table I), the fraction containing compounds **2**–**4** was rechromatographed in M acetic acid, which gave a much better separation. The yields of the pure compounds were: **2**, 70 mg; **3**, 140 mg; and **4**, 59 mg.

Acid hydrolyses. — Each dianhydride (2 mg) was hydrolysed with 0.05M sulphuric acid (2 ml) at 130° for 3 h.

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