Acetolysis of a (4-*O*-methylglucurono)xylan
II.* Characterization of the uronic acids-containing fraction of the acetolyzate

I. ŠIMKOVIC and A. EBRINGEROVÁ

Institute of Chemistry, Slovak Academy of Sciences,
809 33 Bratislava

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Glycuronic and oligoglycuronic acids-containing fraction of the acetolyzate of a (4-*O*-methylglucurono)xylan has been fractionated by elution chromatography on silica gel to yield some of its components as per-*O*-acetyl methyl esters. The substances were characterized by mass spectrometry as such and as corresponding *N*-*p*-toluidyl glycosides, and identified by chromatography (after deesterification and deacetylation). The presence in the acetolyzate of the following substances was confirmed: methyl 1,2,3-tri-*O*-acetyl-4-*O*-methyl-α,β-D-glucopyranuronate, methyl 1,2,3,4-tetra-*O*-acetyl-α,β-D-galactopyranuronate, 1,3,4-tri-*O*-acetyl-2-*O*-(methyl 2,3-di-*O*-acetyl-4-*O*-methyl-α-D-glucopyranosyluronate)-α,β-D-xylopyranose, 1,2,3,3′,4′-penta-*O*-acetyl-2′-*O*-(methyl 2,3-di-*O*-acetyl-4-*O*-methyl-α-D-glucopyranosyluronate)-α,β-xylobiose and its positional isomer, namely 1,3,2′,3′,4′-penta-*O*-acetyl-2-*O*-(methyl 2,3-di-*O*-acetyl-4-*O*-methyl-α-D-glucopyranosyluronate)-α,β-xylobiose.

При помощи колоночной элюентной хроматографии на силикагеле были изолированы некоторые гликуроновые и олигогликуроновые кислоты из кислой фракции ацетолизата (4-*O*-метилглюкуроно)ксилана в виде их метилового эфира пер-*O*-ацетата. Полученные компоненты были характеризованы методом масс-спектрометрии ацетатов и соответствующих *N*-*п*-толуидилгликозидов и были идентифицированы хроматографически после деэтерификации и деацетилирования. Таким путем было доказано присутствие: метил 1,2,3-три-*O*-ацетил-4-*O*-метил-α,β-D-глюкопиранозилуроната, метил 1,2,3,4-тетра-*O*-ацетил-α,β-D-галактопиранозилуроната, 1,3,4-три-*O*-ацетил-2-*O*-(метил-2,3-ди-*O*-ацетил-4-*O*-метил-α-D-глюкопиранозилуронат)-α,β-D-ксилопиранозы, 1,2,3,3′,4′-пента-*O*-ацетил-2′-*O*-(метил-2,3-ди-*O*-ацетил-4-*O*-метил-α-D-глюкопиранозилуронат)-α,β-ксилобиозы и изомера положения 1,3,2′,3′,4′-пента-*O*-ацетил-2-*O*-(метил-2,3-ди-*O*-ацетил-4-*O*-метил-α-D-глюкопиранозилуронат)-α,β-ксилобиозы.

* For Part I see Ref. [4].

In recent years, acetolysis has been widely used as a convenient means of preparing neutral and acidic oligosaccharides from polysaccharides [1, 2]. Compared to other methods of partial depolymerization, acetolysis has the advantage that it often produces well separable mixtures of per-*O*-acetates that can be directly used as intermediates in the synthesis of certain oligosaccharide derivatives.

Partial hydrolysis of (4-*O*-methylglucurono)xylan yields [3] two types of oligosaccharides:

a) β-(1→4)-linked xylooligosaccharides (xylodextrins), representing the constitution of the linear backbone of the macromolecule,

b) oligoxyluronides, reflecting the sequences in the polysaccharide main chain that contains α-(1→2)-linked 4-*O*-methyl-D-glucuronic acid nonreducing end-units.

In the first part of this Series [4] isolation from the acetolyzate of beech wood xylan of a homologous series of α-acetates of neutral xylooligosaccharides (DP 2—5) was reported. In addition to xylooligosaccharides, the acetolyzate furnished a uronic and aldouronic acid-rich fraction. The aim of the present work was to isolate and characterize by mass spectrometry per-*O*-acetates of the components of this acidic fraction.

Experimental

Mass spectra (70 and 12 eV) were obtained with a JMS-D 100 spectrometer at an emission of 300 μA. The temperature at the site of evaporation was, according to the volatility of the substances, 110—370°C. Gas chromatography was performed with a Hewlett—Packard 5700 A research chromatograph, equipped with a column packed with 5% SE-30 on Chromosorb WAW DMCS (100/200 mesh). The temperature was programmed (160—230°C, 4°C/min), and nitrogen was used as the carrier gas. Since, after electron impact, the analyzed per-*O*-acetates do not produce sufficiently intense molecular ions, the acetates were reacted [5] with *p*-toluidine, and the obtained *N*-glycosides produced pronounced [6] molecular ions.

Thin-layer chromatography was performed on glass slides coated with Silica Gel GF_{254} (Merck, Type 60, Darmstadt) and on commercial thin layers of cellulose (Lucefol-Quick, Lachema, Brno). For paper chromatography Whatman No. 1 filter paper was used. Preparative chromatography was done on columns of Silica Gel 60 (Merck, Darmstadt) which, prior to packing, was equilibrated with 40% of the mobile phase. The sample—silica gel ratio was kept at ~1 : 100. Chromatography on silica gel was performed with: *A*. benzene—acetone 6 : 1 and *B*. chloroform—acetone 4 : 1, and paper chromatography was done with *C*. ethyl acetate—acetic acid—water 18 : 7 : 8 and *D*. ethyl acetate—acetic acid—formic acid—water 18 : 3 : 1 : 4. Detection was effected by charring with 5% sulfuric acid in ethanol or by spraying the paper chromatograms with anilinium hydrogen phthalate in acetone, and heating.

Deesterification and deacetylation was performed as described previously [4]. Acidic mono and oligosaccharides were identified (after deesterification and deacetylation) by paper chromatography, by comparison with reference compounds obtained from polysaccharides of hornbeam [7]: 4-*O*-methyl-D-glucuronic acid, 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose, 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)xylobiose, 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose, 4-*O*-(α-D-galactopyranosyluronic acid)-D-xylose, 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose, and commercial (BDH-Chemicals Ltd., Great Britain) D-galacturonic acid. For the determination of α, β ratios by gas chromatography synthetic methyl per-*O*-acetyl-4-*O*-methyl-β-D-glucopyranuronate [8] and per-*O*-acetyl-2-*O*-(methyl 4-*O*-methyl-α-D-glucopyranuronate)-β-D-xylopyranose were used.

Table 1

Yields and chromatographic mobilities of methyl ester per-*O*-acetates of uronic and aldouronic acids

Fraction	Yield %	R_{xyl}	
		β anomer	α anomer
$F_{7/1}$	11.2	1.10[a]	1.05
$F_{7/2}$	3.6	0.93	0.88
$F_{7/3}$	40.4	0.76[a]	0.65
$F_{7/4}$	4.4	–	–
$F_{7/5}$	24.8	0.45	0.36

R_{xyl} — relative to 1,2,3,4-tetra-*O*-acetyl-β-D-xylopyranose.
a) Chromatographically identical with the standard material.

The acidic portion F_7 [4] of the acetolyzate of a (4-*O*-methylglucurono)xylan was extracted with methanol, the extract was deionized with Dowex 50 W (H^+ form) resin, and esterified with diazomethane. The product (250 mg) in chloroform (2 ml) was put on the top of a column (1.6 × 30 cm) of silica gel and eluted with 500 ml portions of 20—6 : 1 (v/v) benzene—acetone mixtures. Sirupy fractions $F_{7/1-5}$ were obtained, the chromatographic mobilities of which in solvent *A* together with yields are summarized in Table 1. They contained mixtures of α and β anomers of which the slower moving α anomers predominated.

Results and discussion

Elution chromatography on a column of silica gel of an acidic fraction of acetolyzate [4] which, prior to chromatography, was rid of higher xylooligosaccharides (DP > 7), deionized and esterified by treatment with diazomethane,

yielded mono- and oligosaccharide fractions $F_{7/1-5}$. The individual components were characterized by mass spectrometry of the corresponding fully acetylated *N*-*p*-toluidyl glycosides [6]. The obtained fractions were sirupy materials and, as in the case of neutral xylooligosaccharides [4] of the same origin, contained anomeric mixtures of per-*O*-acetates of which the α anomers predominated. Further identification of the components was performed by chromatography of the deesterified and deacetylated fractions using, as reference substances, uronic and aldouronic acids isolated and characterized during structural analysis of acidic polysaccharides from hornbeam [7].

The first fraction contained methyl 1,2,3-tri-*O*-acetyl-4-*O*-methyl-α,β-D-glucopyranuronate, as shown by the presence of peaks (terminology according to *Kováčik et al.* [9]) of the Series *A* (*m/z* 289, 229, 187), *C* (*m/z* 201), and *F* (*m/z* 157, 129, 87) in its mass spectrum. The molecular ion peak present in the spectrum of the corresponding *N*-*p*-toluidyl glycoside at *m/z* 395 confirmed the molecular weight of the substance. The 6 : 1 ratio of the anomeric acetates present was calculated from the peak areas on the gas chromatogram. After deacetylation and deesterification the substance cochromatographed with 4-*O*-methyl-D-glucuronic acid.

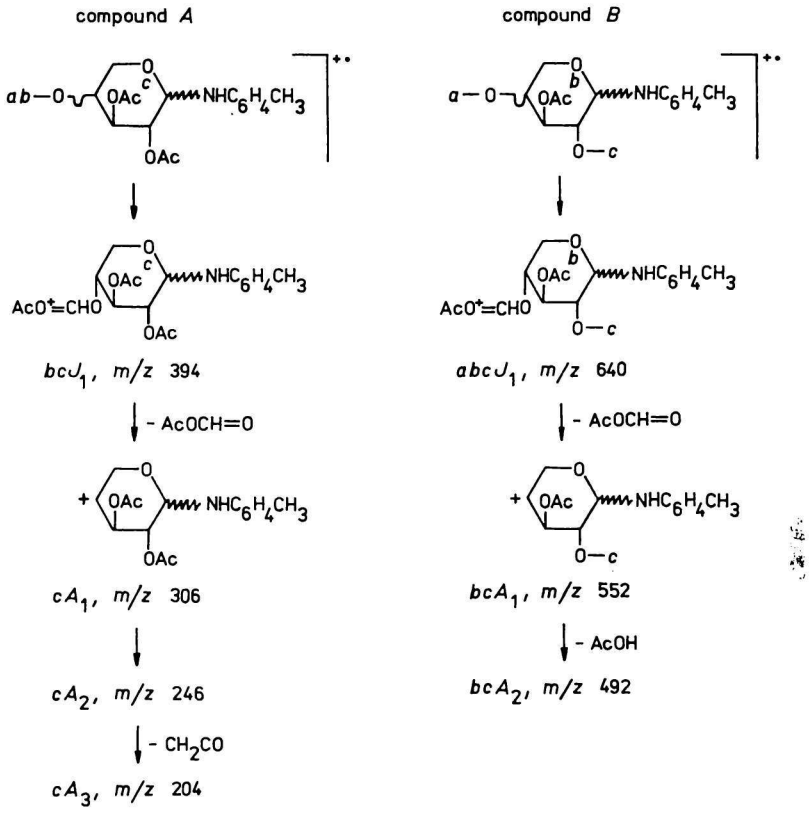
The second fraction contained methyl 1,2,3,4-tetra-*O*-acetyl-α,β-D-galactopyranuronate. Its mass spectrum was qualitatively identical with that [10, 11] of the gluco analogue and the molecular weight of the substance was confirmed by the presence of a pronounced molecular ion peak (*m/z* 423) in the spectrum of the corresponding per-*O*-acetyl-*N*-*p*-toluidyl glycoside. When deesterified and deacetylated the substance showed the same paper chromatographical properties as D-galacturonic acid.

The main fraction of the acidic portion of the acetolyzate was that ($F_{7/3}$, Table 1) containing the derivative of the known structural unit of (4-*O*-methylglucurono)xylans: 1,3,4-tri-*O*-acetyl-2-*O*-(methyl 2,3-di-*O*-acetyl-4-*O*-methyl-α-D-glucopyranosyluronate)-α,β-D-xylopyranose. The $\alpha:\beta$ ratio was 6.6 : 1. The molecular weight of the dimer was confirmed by the $[M+43]^{+\bullet}$ ion peak present in the mass spectrum of the per-*O*-acetate at *m/z* 607, as well as by the molecular ion peak present at *m/z* 611 in the spectrum of the corresponding *N*-*p*-toluidyl glycoside. Ions aA_1 (*m/z* 289, 229, and 187), formed by the cleavage of the glycosidic linkage, confirmed 4-*O*-methyl-D-glucuronic acid as the nonreducing *a* unit of the dimer. Fragmentation according to the *F* Series at the *a* unit [9] gives rise to ions represented by peaks at *m/z* 373 and 331. After deesterification and deacetylation the material was chromatographically indistinguishable from 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose.

Fraction $F_{7/4}$ formed only a small portion of the acidic part of the acetolyzate. It contained several components, as shown by gas chromatography, and mass spectrometry of *N*-*p*-toluidyl glycosides. In addition to the molecular ion peak of

the afore-mentioned dimer, the mass spectrum contained peaks at *m/z* 639, 711, and 653 indicative of the presence of disaccharides consisting of hexuronic acids and a hexose, pentose, and methylpentose. Chromatography of the deesterified and deacetylated $F_{7/4}$ showed the presence of 4-*O*-(α-D-galactopyranosyluronic acid)-D-xylose, 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose, and 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose. Small amounts of these substances have been found in hydrolyzates of wood and hydrolyzates of wood polysaccharides [7, 12]. It has not been proved thus far whether these substances do or do not originate from xylan-contaminating pectic substances or they form structural units in acidic xylans, as some authors suggest [13, 14].

The last fraction ($F_{7/5}$) contained trimers of xylooligouronides, as shown by comparison by chromatography of products of its deesterification and deacetylation with 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)xylobiose. The molecular ion peak (*m/z* 827) present in the mass spectrum of the corresponding *N*-*p*-toluidyl glycoside confirmed the molecular weight of the trimer, present as a mixture of anomers. The same molecular weight was indicated by the peak at *m/z* 721, representing $cabA_1$ ions, present in the spectrum of the per-*O*-acetate. Ions of the series baA_1, aA_1 (aA_2 and aA_3) at *m/z* 505, 289 (229, 187), respectively, confirmed the uronic acid group as a nonreducing end-unit of the trimer, linked to the nonreducing D-xylose unit, the compound being formulated as 1,2,3,3′,4′-penta-*O*-acetyl-2′-*O*-(methyl 2,3-di-*O*-acetyl-4-*O*-methyl-α-D-glucopyranosyluronate)-α,β-xylobiose (*A*). It can be assumed, from the presence of weak peaks of ions of the *cA* Series (*m/z* 259, 199, 157) in the spectrum, that this fraction contained also the isomeric aldotriouronic acid having the uronic acid group linked to the reducing D-xylose residue, namely 1,3,2′,3′,4′-penta-*O*-acetyl-2-*O*-(methyl 2,3-di-*O*-acetyl-4-*O*-methyl-α-D-glucopyranosyluronate)-α,β-xylobiose (*B*). The presence of *B* in the fraction was confirmed also by mass spectrum of the corresponding *N*-*p*-toluidyl glycosides. The mechanism [6] of the fragmentation of *J* ions, formed by cleavage of glycosidic linkages at nonreducing D-xylose units in the two positionally isomeric substances, of which *A* contains the 4-*O*-methyl-D-glucuronic acid group linked to the nonreducing end-unit, is shown in Scheme 1. The isomer *B* produces ions appearing at *m/z* 552 and 402, whereas from the isomer *A* ions at *m/z* 306, 246, and 204 are formed. It follows from the ion abundance in the spectra that isomer *A* is the predominating component. The two isomers could not be separated by paper chromatography (after deacetylation and deesterification). Other authors [15, 16] have suggested that positionally isomeric xylooligouronides (DP > 2) are present in hydrolyzates of (4-*O*-methylglucurono)xylans but this could not be unequivocally proved owing to very similar chromatographical and electrophoretical mobilities of the substances [17].



Scheme 1

Fragmentation of positionally isomeric aldotriouronic acids in fraction $F_{7/5}$

The presented results show that acetolysis of (4-*O*-methylglucurono)xylans is analogous to other acid-catalyzed hydrolytical reactions. The stabilizing effect of the carboxylic group of the uronic acid upon its linkage with D-xylose manifests itself also under acetolysis conditions. This effect is transferred variously also onto glycosidic linkages of the neighbouring D-xylose units and, as a result, in the mixture of higher aldouronic acids the isomers having 4-*O*-methyl-D-glucuronic acid linked to the nonreducing D-xylose end-unit predominate.

It can be concluded that partial acetolysis of (4-*O*-methylglucurono)xylans is not only a means of obtaining neutral and acid oligosaccharides but it is also a possible source of information about the structural features of these wood polysaccharides, which have not been fully clarified thus far. Such are *e.g.* questions about the sites

of branching of the polysaccharide's main chain or about the significance of D-galacturonic acid and L-rhamnose as structural units of the macromolecule.

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