Stereoselective synthesis and ^{13}C NMR spectra of lower oligosaccharides related to arabinoxylan

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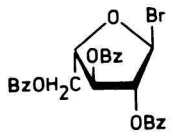
Nucleophiles — 1,2,4-tri-*O*-acetyl-β-D-xylopyranose, methyl 2,4-di-*O*-acetyl-β-D-xylopyranoside, methyl 3,4-di-*O*-benzyl-β-D-xylopyranoside, and methyl 4-*O*-benzyl-β-D-xylopyranoside — were separately condensed with 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl bromide under modified Koenigs—Knorr conditions giving high yields (≈ 90 %) of substituted disaccharides and trisaccharide, respectively. Removal of the protecting groups afforded 3-*O*-α-L-arabinofuranosyl-D-xylopyranose, methyl 3-*O*-α-L-arabinofuranosyl-β-D-xylopyranoside, methyl 2-*O*-α-L-arabinofuranosyl-β-D-xylopyranoside, and methyl 2,3-di-*O*-(α-L-arabinofuranosyl)-β-D-xylopyranoside. ^{13}C NMR spectra of the synthesized compounds are also presented.

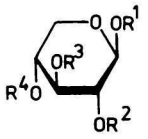
Проведена раздельная конденсация нуклеофилов — 1,2,4-три-*O*-ацетил-β-D-ксилопиранозы, метил-2,4-ди-*O*-ацетил-β-D-ксилопиранозида, метил-3,4-ди-*O*-бензил-β-D-ксилопиранозида и метил-4-*O*-бензил-β-D-ксилопиранозида — с 2,3,5-три-*O*-бензоил-α-L-арабинофуранозилбромидом в модифицированных условиях реакции Кенигса—Кнорра, причем были получены высокие выходы (≈ 90 %) замещенных дисахаридов и трисахарида, соответственно. Устранение защитных групп привело к образованию 3-*O*-α-L-арабинофуранозил-D-ксилопиранозы, метил-3-*O*-α-L-арабинофуранозил-β-D-ксилопиранозида, метил-2-*O*-α-L-арабинофуранозил-β-D-ксилопиранозида и метил-2,3-ди-*O*-(α-L-арабинофуранозил)-β-D-ксилопиранозида. Представлены ^{13}C ЯМР спектры синтезированных соединений.

A basic feature of arabinoxylans representing mainly hemicelluloses of softwoods and annual plants is branching of the backbone created from β-(1 → 4)-linked D-xylopyranosyl residues with α-L-arabinofuranosyl moiety at O-3 resp. O-2 [1, 2]. In order to study structure and properties of these polysaccharides the model disaccharides — 3-*O*-α-L-arabinofuranosyl-D-xylopyranose (*VII*) and its methyl β-glycoside *IX*, methyl 2-*O*-α-L-arabinofuranosyl-β-D-xylopyranoside (*XII*) and the trisaccharide — methyl 2,3-di-*O*-(α-L-arabinofuranosyl)-β-D-xylopyranoside (*XV*) were synthesized (Scheme 1).

We used as a glycosidic agent bromide *I* obtained by treatment of hydrogen



I

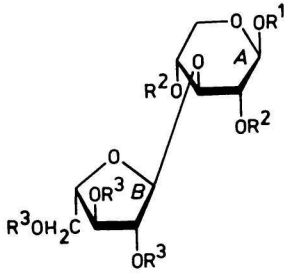


II $R^1 = R^2 = R^4 = Ac$, $R^3 = H$

III $R^1 = Me$, $R^2 = R^4 = Ac$, $R^3 = H$

IV $R^1 = Me$, $R^2 = H$, $R^3 = R^4 = Bn$

V $R^1 = Me$, $R^2 = R^3 = H$, $R^4 = Bn$

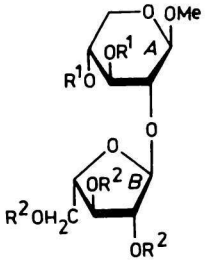


VI $R^1 = R^2 = Ac$, $R^3 = Bz$

VII $R^1 = R^2 = R^3 = H$

VIII $R^1 = Me$, $R^2 = Ac$, $R^3 = Bz$

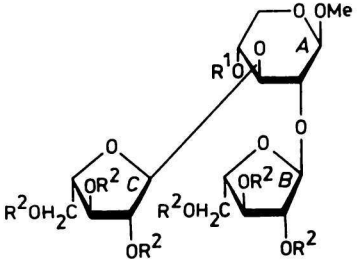
IX $R^1 = Me$, $R^2 = R^3 = H$



X $R^1 = Bn$, $R^2 = Bz$

XI $R^1 = H$, $R^2 = Bz$

XII $R^1 = R^2 = H$



XIII $R^1 = Bn$, $R^2 = Bz$

XIV $R^1 = Bn$, $R^2 = H$

XV $R^1 = R^2 = H$

Ac - acetyl

Bn - benzyl

Bz - benzoyl

Me - methyl

Scheme 1

bromide in acetic acid with methyl 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranoside [3]. Freshly prepared bromide *I* was then applied in twofold excess to the reaction mixtures involving 1,2,4-tri-*O*-acetyl-β-D-xylopyranose (*II*), methyl 2,4-di-*O*-acetyl-β-D-xylopyranoside (*III*) [4], methyl 3,4-di-*O*-benzyl-β-D-xylopyranoside (*IV*) [5], and methyl 4-*O*-benzyl-β-D-xylopyranoside (*V*) [6]. Mercuric cyanide was used as a catalyst and acid scavenger.

Since 1,2-*trans* glycosidic bonds are created first of all in nonpolar solvents [7, 8], the synthesis of disaccharide *VIII* was examined in benzene, dichloromethane, and chloroform. It was found that the highest stereoselectivity of condensation reaction in favour of creating α-(1 → 3)-glycosidic bond between bromide *I* and nucleophile *III* was reached in benzene. After 5 h at room temperature the reaction mixture in this solvent contained as much as 95 % of the expected methyl 2,4-di-*O*-acetyl-3-*O*-(2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl)-β-D-xylopyranoside (*VIII*). In respect to high yield of protected disaccharide *VIII*, condensations of bromide *I* with nucleophiles *II*, *IV*, and *V* were performed at the same conditions.

It is also necessary to emphasize that during all four syntheses of oligosaccharides *VI*, *VIII*, *X*, and *XIII* no traces of disaccharides or trisaccharide containing β-linked L-arabinofuranosyl residue were isolated from the reaction mixtures.

The compounds *VI*, *VIII*, *X*, and *XIII* were purified using column chromatography on silica gel with linear gradient elution. Deacylation of *VI* and *VIII* by sodium methoxide in methanol afforded the title disaccharides *VII* and *IX*, respectively; deacylation and catalytic hydrogenation of *X* and *XIII* gave *XII* and *XV*

Compounds *VI*—*VIII*, *X*—*XV* that were hitherto unknown were characterized by physical constants, and their structures confirmed by analysis of ^{13}C NMR spectra (Table 1).

Experimental

Melting points were determined on a Kofler hot stage. Optical rotations (D, 22 °C, $\varrho = 10\,\mathrm{g\,dm^{-3}}$, if not stated otherwise) were measured with a Perkin—Elmer Model 141 automatic polarimeter. All reactions were monitored by TLC on Silica gel G and preparative chromatography was performed by gradient elution from columns of dry-packed Silica gel 60 (Merck, Darmstadt) which, prior to packing, had been equilibrated with 40 % of the mobile phase using: benzene—acetone (volume ratio = 15 : 1 (*A*), 20 : 1 (*B*), 30 : 1 (*C*), and 35 : 1 (*D*)); chloroform—methanol (volume ratio = 6 : 1 (*E*)); benzene—ethyl acetate (volume ratio = 30 : 1 (*F*)) and chloroform—acetone (volume ratio = 10 : 1 (*G*)). The detection was effected by charring with 5 % sulfuric acid in ethanol.

^{13}C NMR spectra were measured in $CDCl_3$ (compounds *VI*, *VIII*, *X*, *XI*, *XIII*, and

Table 1

^{13}C NMR chemical shifts (δ/ppm) for oligosaccharides *VI*—*XV*

Compound	Ring	C-1	C-2	C-3	C-4	C-5	OMe
VI	*A*	92.32	70.17	76.73	69.46	62.96	
	B	106.69	82.26	77.19	81.87	63.61	
VII	*A*	97.72	75.24	83.17	69.20	66.21	
	B	109.48	82.39	77.71	85.18	62.43	
VIII	*A*	101.88	71.67	77.06	70.04	62.24	56.53
	B	106.88	82.19	77.84	81.80	63.67	
IX[b]	*A*	105.06	74.20	83.04	69.13	66.21	58.47
	B	109.42	82.45	77.77	85.25	62.50	
X	*A*	103.50	78.43[a]	84.24	78.17[a]	63.89	56.73
	B	105.58	81.99	77.20	80.95	63.79	
XI	*A*	102.25	76.46	74.43	69.50	63.91	56.49
	B	106.00	82.74	77.45	80.78	63.65	
XII	*A*	104.14	77.99	76.75	70.33	66.09	58.35
	B	109.46	82.24	79.18	85.67	62.48	
XIII	*A*	103.72	77.42	78.77	77.22	63.72	56.73
	B	105.95	81.66[a]	77.97	81.88	64.17	
	C	105.95	81.99[a]	77.82	81.50	63.50	
XIV	*A*	103.91	77.59	78.15	76.87	63.91	58.31
	B	109.69	82.34	80.53[a]	85.71	62.43	
	C	109.69	82.69	79.32[a]	84.93	61.61	
XV	*A*	104.05	77.73	78.09	69.20	65.85	58.39
	B	109.63	82.39	78.97	85.62	62.44	
	C	109.63	83.11	78.97	85.17	62.44	

a) The assignments may be reversed; *b*) the chemical shifts are in agreement with Ref. [12].

XIV, internal standard TMS) and in D_2O (compounds *VII*, *IX*, *XII*, and *XV*, internal standard methanol, $\delta_{TMS} = 50.15$ ppm) at room temperature on a Bruker AM-300 and Jeol FX-100 spectrometers, respectively.

^{13}C Resonances with small differences (less than 1.5 ppm) of chemical shifts (*e.g.* C-2 and C-4 atoms in compounds *VI* and *VIII*, atoms C-5 in compounds *X*, *XI*, *XIII*, and *XIV*) were assigned using semiselective INEPT experiment [9]. Soft pulses were applied on the preselected protons and corresponding long-range ^{13}C signals were detected. We set the delays between pulses to 24 ms and 28 ms, respectively, in that pulse sequence, *i.e.* optimum for approximately 7 Hz coupling constants.

In order to analyze the spectrum of the compound *XIII* the two-dimensional heteronuclear correlation experiment was performed. An initial data matrix of 256 × 1024 points represented spectral widths (F1 × F2 domains) of 500 × 1600 Hz. 512 transients were accumulated in each FID (128 FIDs in the experiment) using 19 μs for ^{13}C and 41 μs for ^{1}H $\pi/2$ pulses, respectively. A weighted function was used prior to Fourier transform (shifted sine-bell in F2, Gaussian in F1).

Microanalyses were performed with a Perkin—Elmer Model 240 automatic analyzer.

Benzene and toluene were dried with sodium and calcium hydride, respectively, and freshly distilled. Solutions were dried with anhydrous sodium sulfate and concentrated at 40 °C and 2 kPa.

*1,2,4-Tri-O-acetyl-β-*D*-xylopyranose (II)*

1,2,4-Tri-*O*-acetyl-3-*O*-benzyl-α,β-D-xylopyranose [4] (3 g) was hydrogenolyzed in acetone—methanol ($\varphi_r = 1:4$, 150 cm^3) at room temperature over 5 % Pd/C (0.3 g) for 5 h. TLC (solvent *A*) then showed only the presence of product *II* ($R_f = 0.1$). After conventional work-up compound *II* crystallized from acetone—diethyl ether (1.9 g, 84 %) and after the second recrystallization showed m.p. = 147—150 °C and [α] (chloroform) = −22°. Ref. [10] gives m.p. = 138 °C and [α] (20 °C, chloroform) = −21°

For $C_{11}H_{16}O_8$ ($M_r = 276.24$) w_i(calc.): 47.82 % C, 5.84 % H; w_i(found): 47.79 % C, 5.93 % H.

*1,2,4-Tri-O-acetyl-3-O-(2,3,5-tri-O-benzoyl-α-*L*-arabinofuranosyl)-β-*D*-xylopyranose (VI)*

A solution of bromide *I* (freshly prepared from methyl 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranoside [3]) (3.45 g; 7.24 mmol) in the minimum amount of benzene was added to a mixture of *II* (1 g; 3.62 mmol), mercuric cyanide (0.92 g; 3.64 mmol) in benzene (50 cm^3) and the resulting mixture was stirred with the exclusion of atmospheric moisture at room temperature for 5 h. TLC (solvent *B*) then showed only traces of nucleophile *II* ($R_f = 0.05$), the presence of the disaccharide *VI* ($R_f = 0.4$) and the hydrolysis product ($R_f = 0.3$) of *I*. Small amounts of by-products ($R_f > 0.5$) were also present. The mixture was worked-up [11], and the product was subjected to chromatography, using linear gradient elution (solvents $C \rightarrow D$). Crystallization from methanol gave *VI* (2.4 g, 92 %), m.p. = 119—122 °C, [α] (chloroform) = −3.8°.

For $C_{37}H_{36}O_{15}$ ($M_r = 720.66$) w_i(calc.): 61.66 % C, 5.04 % H; w_i(found): 61.50 % C, 5.13 % H.

*3-O-α-*L*-Arabinofuranosyl-*D*-xylopyranose (VII)*

1 M methanolic solution of sodium methoxide (1 cm^3) was added to a solution of *VI* (2 g) in methanol (100 cm^3) and the solution was kept for 1 h at room temperature. Then TLC showed deacylation to be complete and the presence of product *VII* ($R_f = 0.3$, solvent *E*). The solution was neutralized with Dowex 50 W (H^+) resin, filtered, and concentrated. The residue was freed from methyl benzoate by chromatography and crystallized from ethanol to give *VII* (0.65 g, 83.3 %), m.p. = 138—139 °C, [α] (water) = = −64°.

For $C_{10}H_{18}O_9$ ($M_r = 282.24$) w_i(calc.): 42.55 % C, 6.43 % H; w_i(found): 42.41 % C, 6.56 % H.

Methyl 2,4-di-O-acetyl-3-O-(2,3,5-tri-O-benzoyl-α-L-arabinofuranosyl)-β-D-xylopyranoside (VIII)

Compound *III* [4] (1.5 g; 6 mmol) was condensed with *I* (prepared from methyl 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranoside (5.75 g; 12 mmol) by treatment of 40 % hydrogen bromide (25 cm^3) in acetic acid for 30 min) as described for preparation of *VI*.

Conventional isolation gave amorphous *VIII* (4 g, 95.7 %), [α] (chloroform) = −17°

For $C_{36}H_{36}O_{14}$ (M_r = 692.65) w_i(calc.): 62.42 % C, 5.24 % H; w_i(found): 62.33 % C, 5.30 % H.

Methyl 3-O-α-L-arabinofuranosyl-β-D-xylopyranoside (IX)

Deacetylation and debenzoylation of compound *VIII* (3 g), as described for the preparation of *VII*, afforded disaccharide *IX* (1.15 g, 89.8 %, R_f = 0.35, solvent *E*). Crystallization from acetone and recrystallization from methanol gave material having m.p. = 135—137 °C, [α] (water) = −127.5°.

For $C_{11}H_{20}O_9$ (M_r = 296.27) w_i(calc.): 44.59 % C, 6.80 % H; w_i(found): 44.61 % C, 6.94 % H. Ref. [12] gives [α] (20 °C, water) = −113°; for $C_{11}H_{20}O_9 \cdot 1.5\,H_2O$ (M_r = = 323.29) w_i(calc.): 40.87 % C, 7.17 % H; w_i(found): 40.53 % C, 6.86 % H.

Methyl 3,4-di-O-benzyl-2-O-(2,3,5-tri-O-benzoyl-α-L-arabinofuranosyl)-β-D-xylopyranoside (X)

To the mixture of nucleophile *IV* [5] (2 g; 5.8 mmol), mercuric cyanide (1.5 g; 5.9 mmol), and benzene (50 cm^3) bromide *I* (prepared from methyl 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranoside (5.5 g; 11.5 mmol) [3]) was added and the reaction mixture was stirred at room temperature with exclusion of atmospheric moisture for 2 h. After work-up the reaction mixture contained mainly disaccharide *X* (R_f = 0.65) and the hydrolysis product of *I* (R_f = 0.35) (solvent *B*) which were separated by column chromatography on silica gel (solvent *F*). The product *X* (4.2 g, 91.7 %) was isolated as a colourless sirup, [α] (chloroform) = −1.95°.

For $C_{46}H_{44}O_{12}$ (M_r = 788.81) w_i(calc.): 70.03 % C, 5.62 % H; w_i(found): 69.88 % C, 5.79 % H.

Methyl 2-O-(2,3,5-tri-O-benzoyl-α-L-arabinofuranosyl)-β-D-xylopyranoside (XI)

A mixture of disaccharide *X* (3.5 g), 5 % Pd/C (0.5 g), acetone—methanol (φ_r = = 1 : 10, 300 cm^3) was stirred at room temperature under hydrogen for 4 h, and then TLC (solvent *F*) showed the absence of *X* (R_f = 0.65). The mixture was processed in a

conventional manner and partially substituted disaccharide *XI* (2.5 g, 92.6 %, $R_f = 0.3$, solvent *G*) then crystallized from ether—hexane ($\varphi_r = 1:2$), m.p. = 68—70 °C, [α] (chloroform) = −12.4°.

For $C_{32}H_{32}O_{12}$ ($M_r = 608.58$) w_i(calc.): 63.15 % C, 5.30 % H; w_i(found): 62.93 % C, 5.30 % H.

Methyl 2-O-α-L-arabinofuranosyl-β-D-xylopyranoside (XII)

Debenzoylation of *XI* (2 g), as described for preparation of *VII*, afforded *XII* (0.85 g, 87.6 %, $R_f = 0.4$, solvent *E*) as an amorphous material, [α] (water) = −112°.

For $C_{11}H_{20}O_9$ ($M_r = 296.27$) w_i(calc.): 44.59 % C, 6.80 % H; w_i(found): 44.35 % C, 7.05 % H.

Methyl 2,3-di-O-(2,3,5-tri-O-benzoyl-α-L-arabinofuranosyl)-4--O-benzyl-β-D-xylopyranoside (XIII)

Methyl 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranoside (7.5 g; 15.7 mmol) was converted to bromide *I* and added to a mixture of *V* (1 g; 3.9 mmol), mercuric cyanide (2 g; 7.9 mmol) in benzene (50 cm^3) and the reaction mixture was stirred at room temperature with the exclusion of atmospheric moisture for 1 h. TLC then showed (solvent *B*) the absence of *V* ($R_f = 0.05$), trisaccharide *XIII* ($R_f = 0.6$), the hydrolysis product of *I* ($R_f = 0.35$) and a small amount of by-products ($R_f > 0.65$). The reaction mixture was worked-up conventionally, purified by column chromatography (solvent *F*), and yielded colourless sirup *XIII* (4 g, 89 %), [α] (chloroform) = −6°.

For $C_{65}H_{58}O_{19}$ ($M_r = 1143.11$) w_i(calc.): 68.29 % C, 5.11 % H; w_i(found): 68.18 % C, 5.15 % H.

Methyl 2,3-di-O-(α-L-arabinofuranosyl)-4-O-benzyl-β-D-xylopyranoside (XIV)

Debenzoylation of *XIII* (3.5 g) as described for the preparation of *VII*, afforded *XIV* (1.3 g, 81.8 %, $R_f = 0.35$, solvent *E*) which crystallized from acetone, m.p. = 139—141 °C, [α] (water) = −126.8°.

For $C_{23}H_{34}O_{13}$ ($M_r = 518.50$) w_i(calc.): 53.27 % C, 6.61 % H; w_i(found): 53.54 % C, 6.70 % H.

Methyl 2,3-di-O-(α-L-arabinofuranosyl)-β-D-xylopyranoside (XV)

A mixture of *XIV* (1 g) and 5 % Pd/C (0.2 g) in methanol (100 cm^3) was stirred at room temperature under hydrogen for 4 h. Trisaccharide *XV* was obtained (0.75 g, 90.8 %, $R_f = 0.2$, solvent *E*) as a colourless sirup, [α] (water) = −147.4°.

For $C_{16}H_{28}O_{13}$ ($M_r = 428.38$) w_i(calc.): 44.86 % C, 6.59 % H; w_i(found): 44.67 % C, 6.88 % H.

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