Sequential synthesis and ^{13}C NMR spectra of methyl 3-*O*- and 2-*O*-(β-D-xylobiosyl)-β-D-xylopyranosides

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Condensation of 2,3-di-*O*-acetyl-4-*O*-benzyl-α, β-D-xylopyranosyl bromide (*I*) with methyl 2,4-di-*O*-acetyl-β-D-xylopyranoside (*II*) yielded a mixture of methyl tetra-*O*-acetyl-4′-*O*-benzyl-α- and -β-(1→3)-D-xylobiosides. Therefrom, the disaccharide *V* having a free hydroxyl group at C-4′ was isolated by chromatography after debenzylation. Then it was condensed with 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide (*VIII*). The resulting peracetylated trisaccharides *IX* and *X* isomeric in the site of the interglycosidic linkage of the nonreducing end yielded after deacetylation the title methyl 3-*O*-(β-D-xylobiosyl)-β-D-xylopyranoside (*XIV*) and its 4′-*O*-α-glycosidically bound isomer *XIII*. The same synthetic principle was applied to the synthesis of methyl 2-*O*-(β-D-xylobiosyl)-β-D-xylopyranoside (*XVI*) using methyl 3,4-di-*O*-acetyl-β-D-xylopyranoside (*III*) as a nucleophile. All the compounds reported herein were obtained in crystalline state. Their ^{13}C-n.m.r. spectra are also presented and interpreted.

Конденсацией 2,3-ди-*O*-ацетил-4-*O*-бензил-α,β-D-ксилопиранозилбромида (*I*) с метил-2,4-ди-*O*-ацетил-β-D-ксилопиранозидом (*II*) была получена смесь метил-тетра-*O*-ацетил-4′-*O*-бензил-α- и -β-(1→3)-D-ксилобиозидов, из которой нуклеофил *V* хроматографически изолировался после дебензилирования. Дисахарид *V* со свободной ОН группой на С-4′ был сконденсирован с 2,3,4-три-*O*-ацетил-α-D-ксилопиранозилбромидом (*VIII*), а из образовавшихся перацетатов трисахаридов *IX* и *X*, изомерных в месте межгликозидной связи нередуцирующего конца после деацетилирования образовался искомый метил-3-*O*-(β-D-ксилобиозил)-β-D-ксилопиранозид (*XIV*) и его 4′-*O*-α-гликозидно связанный изомер *XIII*. Подобным образом был получен метил-2-*O*-(β-D-ксилобиозил)-β-D-ксилопиранозид (*XVI*) при применении в качестве нуклеофила метил-3,4-ди-*O*-ацетил-β-D-ксилопиранозида (*III*). Все упомянутые соединения были получены в кристаллическом виде и представлены их ^{13}C-ЯМР спектры.

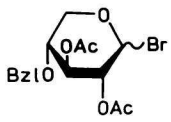
One of the structural features characteristic of xylan type polysaccharides is the branching of some linear chain units in positions C-3 and/or C-2 by β-glycosidical-

ly bound units of D-xylose or β-(1→4) xylooligosaccharides (the so-called xylodextrins) [1]. In study of the structure and the properties of the xylan polysaccharides are especially useful model substances — synthetically prepared lower xylooligosaccharides and their methyl β-glycosides. The β-glycosidically bound aglycone thus imitates the situation in the polysaccharide backbone chain. In this paper we report the synthesis of methyl 3-*O*- and 2-*O*-[4-*O*-(β-D-xylopyranosyl)-β-D-xylopyranosyl]-β-D-xylopyranosides (*XIV* and *XVI*) which both supplement the series of the hitherto prepared methyl β-xylotriosides of the xylan type [2—7].

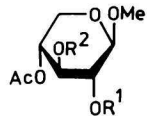
2,3-Di-*O*-acetyl-4-*O*-benzyl-α,β-D-xylopyranosyl bromide [7] (*I*), as the first glycosylating agent in this reaction sequence, was prepared by treatment of 1,2,3-tri-*O*-acetyl-4-*O*-benzyl-β-D-xylopyranose [8] with a dichloromethane solution of hydrogen bromide. Because of its high reactivity, the bromide *I* was further used without isolation immediately after preparation. It was added in 2.5-fold excess to the nucleophiles, *i.e.* methyl 2,4- and 3,4-di-*O*-acetyl-β-D-xylopyranosides [9, 10] (*II* and *III*, respectively) in acetonitrile under the presence of mercury(II) cyanide. However, it is necessary that the bromide *I* be freed from residual hydrogen bromide before adding to the glycosylation reaction mixture. Otherwise, when using equimolar amounts of $Hg(CN)_2$ excessive amounts of HBr would cause migration of the acetyl groups in the partially acetylated dissaccharides *II* and *III* leading thus to complicated, difficult to separate reaction mixtures. This observation is in agreement with the previous knowledge [11] on acid-induced migration of acyl groups in partially acetylated saccharides. Considerable accesses of the bromide *I* were required to compensate losses due to side reactions and in order to properly utilize the nucleophiles *II* and *III*. From each of the two reaction mixtures, unresolved pairs of anomeric disaccharides, *i.e.* methyl 2,4-di-*O*-acetyl-3-*O*-(2,3-di-*O*-acetyl-4-*O*-benzyl-α- and -β-D-xylopyranosyl)-β-D-xylopyranosides and methyl 3,4-di-*O*-acetyl-2-*O*-(2,3-di-*O*-acetyl-4-*O*-benzyl-α- and -β-D-xylopyranosyl)-β-D-xylopyranosides, were isolated using column chromatography on silica gel with linear gradient elution. Debenzylation and subsequent chromatography of the above-mentioned isomeric mixtures afforded, on the one hand, the pure isomeric disaccharides *IV* and *V* in the ratio 1:1.5 and *VI* and *VII* in the ratio 1:2, on the other hand.

The desired peracetylated trisaccharides *IX* and *X*, as well as the analogous *XI* and *XII*, were obtained in high total yields (over 90 %) in the ratio 1:3 *via* condensation of the nucleophiles *V* and *VII* with acetobromoxylose [12] in an acetonitrile solution and under the presence of mercury(II) cyanide as a catalyst and acid scavenger. Their deacetylation afforded the title compounds *XIV* and *XVI* and their 4′-*O*-α-glycosidically bound isomers *XIII* and *XV*

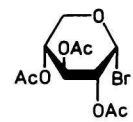
Since yields of the pure compounds obtained from poorly separable mixtures substantially depended on a separation efficiency achieved, the preparative



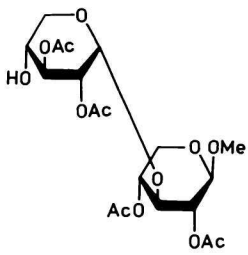
I



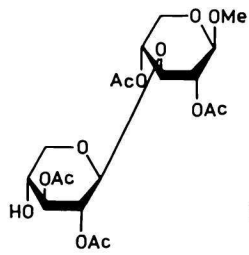
II R^1 = Ac, R^2 = H
III R^1 = H, R^2 = Ac



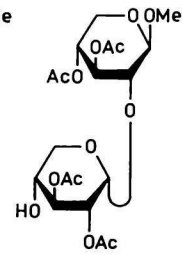
VIII



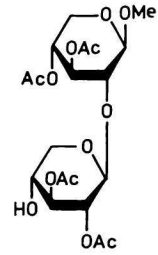
IV



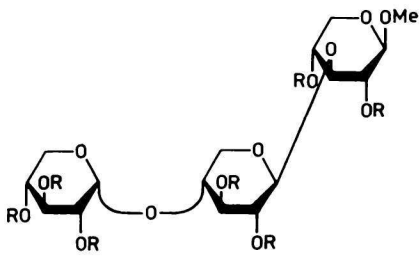
V



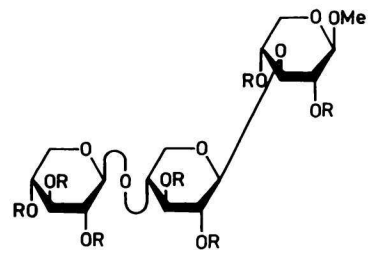
VI



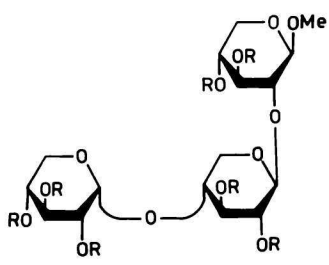
VII



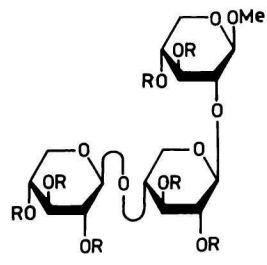
IX R = Ac
XIII R = H



X R = Ac
XIV R = H



XI R = Ac
XV R = H



XII R = Ac
XVI R = H

chromatography was in most cases repeated. All the newly prepared compounds were obtained in crystalline state and characterized by elementary physicochemical constants. Configurations of the interglycosidic linkages were assigned tentatively according to the values of specific optical rotation. The structure was confirmed by analysis of their ^{13}C-n.m.r. spectra.

The ^{13}C-n.m.r. data of all the prepared di- and trisaccharides are given in Table 1. The individual signals in spectra of the disaccharides IV—VII were assigned to

Table 1

^{13}C NMR chemical shifts δ_c/ppm of the compounds *IV—VII* and *IX—XVI*

Compound	Ring	C-1	C-2	C-3	C-4	C-5	OMe
IV	C	101.68	71.28	75.56	70.69	61.53	56.46
	C′	95.96	71.08	73.29	69.00	61.92	
V	C	100.96	70.76	76.67	69.07	61.01	
	C′	100.64	70.11	74.78	68.22	64.71	56.07
VI	C	104.15	76.28	72.58	69.72	61.92	56.85
	C′	95.77	70.37	73.68	69.20	62.50	
VII	C	102.40	76.80	73.10	69.39	61.92	56.79
	C′	100.90	70.50	75.24	68.22	64.97	
IX	C	100.84	70.76	76.41	69.00	60.95	56.07
	C′	100.71	70.76	73.16[a]	73.68[a]	63.80	
	C″	96.35	71.08	69.00	69.00	58.74	
X	C	100.90	70.69	76.41	68.94	60.88	56.07
	C′	100.90	70.69	72.32	74.72	62.76	
	C″	99.60	70.43	70.43	68.29	61.53	
XI	C	102.60	77.08	73.50	69.14	62.24	56.96
	C′	101.10	71.61	74.22[a]	73.05[a]	64.06	
	C″	96.48	70.96	69.14	69.60	58.85	
XII	C	102.33	76.93	73.31	69.46	62.12	56.85
	C′	101.23	71.15	72.36	74.79	62.76	
	C″	99.67	70.37	70.37	68.35	61.53	
XIII	C	104.99	73.89	85.19	69.02	66.10	58.50
	C′	104.54	74.21	75.77	79.28	65.45	
	C″	101.35	72.85	74.41	70.58	62.85	

Table 1 (Continued)

Compound	Ring	C-1	C-2	C-3	C-4	C-5	OMe
XIV	C	105.08	73.89	85.09	68.94	66.08	58.46
	C′	104.67	74.54	74.93	77.67	64.19	
	C″	103.19	74.02	76.89	70.44	66.47	
XV	C	104.60	81.81	76.55	70.26	65.97	58.18
	C′	103.76	74.22	75.64	79.22	65.38	
	C″	101.29	72.85	74.34	70.51	62.85	
XVI	C	104.74	81.74	76.60	70.30	65.95	58.22
	C′	103.76	74.52	74.85	77.64	64.19	
	C″	103.18	74.07	76.86	70.50	66.53	

a) Assignment may be also reversed.

the corresponding carbon atoms on the basis of comparison with analogous peracetylated xylobioses [13] and methyl di-*O*-acetyl-β-D-xylopyranosides [14], taking into consideration the contributions of the D-xylosyl moiety [15]. Chemical shifts in the peracetylated trisaccharides *IX*—*XII* were ascribed to the corresponding carbon atoms after comparing with the disaccharides *IV*—*VII* and peracetylated xylobioses. Quite analogously, signal assignments in methyl xylosides *XIII*—*XVI* were done with the aid of isomeric methyl xylobiosides [15].

Experimental

Melting points were determined on a Kofler hot stage. Optical rotation measurements were carried out at 22 °C and $\varrho \doteq 10$ g dm^{-3} on a Perkin—Elmer automatic polarimeter, Model 141. Thin-layer chromatography (t.l.c.) on Silica gel G and preparative chromatography on Silica gel 60 (Merck A.G., Darmstadt) preconditioned with 40 % of mobile phase were performed in the following solvent systems: carbon tetrachloride—ethyl acetate (volume ratio = 4 : 1 (*A*), 3 : 1 (*B*), 2 : 1 (*C*), 1.5 : 1 (*D*), 1 : 1 (*E*)) and chloroform—methanol (volume ratio = 4 : 1 (*F*)). The spots on t.l.c. chromatograms were visualized by spraying with 5 volume % sulfuric acid in ethanol and heating until permanent staining.

^{13}C-N.m.r. spectra were measured at room temperature in $CDCl_3$ (the compounds *IV*—*VII*, *IX*—*XII*, internal standard TMS) and in D_2O (the compounds *XIII*—*XVI*, internal standard methanol, $\delta_{r.TMS} = 50.15$ ppm) using Jeol JMN FX-60 and FX-100 instruments.

Dichloromethane solution of HBr (ϱ(HBr) = 0.08 g cm^{-3}, as determined by weighing) was prepared in the way described earlier [10]. Toluene was refluxed over sodium hydride

and used freshly distilled. Acetonitrile was dried with calcium hydride and then distilled. Dichloromethane was dried with phosphorus pentoxide and then distilled. Solutions in organic solvents were dried over anhydrous sodium sulfate and concentrated at 40 °C and 2 kPa.

Microanalyses were performed on an automatic analyzer Perkin—Elmer, Model 240.

Methyl 2,4-di-O-acetyl-3-O-(2,3-di-O-acetyl-α- and -β-D--xylopyranosyl)-β-D-xylopyranoside (IV and V)

Dichloromethane solution of hydrogen bromide (75 cm^3) was added to the solution of 1,2,3-tri-*O*-acetyl-4-*O*-benzyl-β-D-xylopyranose [8] (11 g; 30 mmol) in dichloromethane (20 cm^3), and the mixture was kept at room temperature for 30 min. After concentrating to a thick sirup (three times with addition of toluene), the resulting glycosyl halide *I* was dissolved in a small amount of acetonitrile (*ca.* 10 cm^3) and added to a stirred solution of methyl 2,4-di-*O*-acetyl-β-D-xylopyranoside [9] (*II*, 3 g; 12.09 mmol) and mercury(II) cyanide (3.8 g; 15 mmol) in acetonitrile (40 cm^3). After 1 h stirring at room temperature, t.l.c. in the solvent system *B* (twofold development) showed the absence of the starting *II* ($R_f = 0.25$). At the same time, there could be detected on the chromatogram an unresolved pair of the desired disaccharides — methyl 2,4-di-*O*-acetyl-3-*O*-(2,3-di-*O*-acetyl--4-*O*-benzyl-α- and -β-D-xylopyranosyl)-β-D-xylopyranosides $R_f = 0.45$) — as well as the product of hydrolysis of the bromide ($R_f = 0.4$). Small amounts of some unidentified by-products were also present ($R_f > 0.5$). After working up the mixture in the usual way, it was separated using column chromatography with a linear gradient elution (1200 g of silica gel, solvent systems $A \rightarrow B$). The collected fraction containing mostly the reaction product ($R_f = 0.45$, 7.5 g) was hydrogenated in the solvent mixture acetone—methanol (250 cm^3, volume ratio = 1:4) over 5 % Pd/C (1 g) at room temperature and atmospheric pressure. After 3 h reaction time, t.l.c. in the solvent system *E* (twofold development) showed a complete conversion of the glycosidation reaction product ($R_f = 0.55$) to the desired *IV* and *V* ($R_f = 0.35$ and 0.3) in the ratio approximately 2:3. After working up in the usual manner, the mixture was separated using column linear gradient elution chromatography (800 g of silica gel, solvent systems $D \rightarrow E$). The first collected fraction, containing mainly the product of $R_f = 0.35$, gave after crystallization from methanol the chromatographically pure *IV* (yield = 1.35 g (24.1 %)), which after recrystallization exhibited m.p. = 158—159 °C and $[\alpha]_D = +44°$ ($CHCl_3$).

For $C_{19}H_{28}O_{13}$ ($M_r = 464.41$) w_i(calculated): 49.13 % C, 6.08 % H; w_i(found): 49.27 % C, 6.20 % H.

A further elution yielded the fraction containing mainly the disaccharide *V*, which crystallized from ether. So was obtained the chromatographically pure *V* (yield = 2 g (35.6 %)), which after recrystallization gave m.p. = 140—141 °C and $[\alpha]_D = -92°$ ($CHCl_3$). Elemental analysis found $w_i = 49.31$ % C and 6.23 % H.

Further crops of the disaccharides *IV* (yield = 0.35 g (6.2 %) — overall yield = 1.7 g (30.3 %)) and *V* (yield = 0.6 g (10.7 %) — overall yield = 2.6 g (46.3 %)) were recovered by rechromatography of the mixture fractions and the mother liquors from the crystallizations.

Methyl 3,4-di-O-acetyl-2-O-(2,3-di-O-acetyl-α- and -β-D-xylopyranosyl)-β-D-xylopyranosides (VI and VII)

The preparation of the bromide *I* and its subsequent condensation with methyl 3,4-di-*O*-acetyl-β-D-xylopyranoside [10] (*III*) were performed in the same amounts and under the same conditions as described above (preparation of *IV* and *V*). The product consisting of an unseparable mixture of two disaccharides — methyl 3,4-di-*O*-acetyl--2-*O*-(2,3-di-*O*-acetyl-4-*O*-benzyl-α- and -β-D-xylopyranosyl)-β-D-xylopyranosides ($R_f = 0.35$, solvent system *B*) — was isolated from the reaction mixture using column linear gradient elution chromatography. Hydrogenolysis of this mixture afforded a mixture of poorly but still separable disaccharides *VI* and *VII*. After twofold column gradient elution chromatography (firstly — 800 g, secondly — 400 g of silica gel, solvent mixtures $D \rightarrow E$), the following crystalline disaccharides were obtained: *VI* — yield = 1.55 g (27.6 %), m.p. = 172.5—174.5 °C after the second recrystallization from ethanol, $[\alpha]_D = +95.2°$ ($CHCl_3$). For $C_{19}H_{28}O_{13}$ ($M_r = 464.41$) w_i(calculated): 49.13 % C, 6.08 % H; w_i(found): 49.13 % C, 6.31 % H; *VII* — yield = 3.05 g (54.4 %), m.p. = 131—132 °C after the second recrystallization from ether, $[\alpha]_D = -60.3°$ ($CHCl_3$). Elemental analysis found $w_i = 49.11$ % C and 6.16 % H.

Methyl 2,4-di-O-acetyl-3-O-[2,3-di-O-acetyl-4-O-(2,3,4-tri-O--acetyl-α- and -β-D-xylopyranosyl)-β-D-xylopyranosyl]--β-D-xylopyranosides (IX and X)

Tri-*O*-acetyl-α-D-xylopyranosyl bromide [12] (*VIII*, 3.1 g; 9.14 mmol) was added to the mixture of *V* (1.7 g; 3.66 mmol) and mercury(II) cyanide (1.15 g; 4.57 mmol) in acetonitrile (15 cm^3). The resulting mixture was stirred with exclusion of air humidity at laboratory temperature during 1 h. Then t.l.c. in the solvent system *C* (twofold development) showed disappearance of the starting disaccharide *V* ($R_f = 0.15$) and the presence predominantly of the glycosylation product, that is a mixture of *IX* and *X* ($R_f = 0.3$ and 0.25), trace amounts of the bromide hydrolysis product ($R_f = 0.2$), and some other unidentified by-products ($R_f > 0.45$). The mixture was worked up in the usual manner and fractionated by column linear gradient elution chromatography (550 g of silica gel, solvent systems $C \rightarrow D$). The fractions containing the pure trisaccharides *IX* and *X* were collected and the remaining unresolved material was rechromatographed.

The compound *IX*, yield = 0.6 g (22.7 %), gave m.p. = 189—190 °C after the second recrystallization from methanol and $[\alpha]_D = -13.2°$ ($CHCl_3$). For $C_{30}H_{42}O_{20}$ ($M_r = 722.64$) w_i(calculated): 49.86 % C, 5.86 % H; w_i(found): 49.76 % C, 5.88 % H.

The compound *X*, yield = 1.85 g (69.9 %), gave m.p. = 178—179 °C after the second recrystallization from a mixture acetone—ether (volume ratio = 1:3) and $[\alpha]_D = -101.2°$ ($CHCl_3$). Elemental analysis found $w_i = 49.76$ % C and 5.95 % H.

Methyl 3,4-di-O-acetyl-2-O-[2,3-di-O-acetyl-4-O-(2,3,4-tri-O-acetyl-α- and -β-D-xylopyranosyl)-β-D-xylopyranosyl]-β-D-xylopyranosides (XI and XII)

Acetobromoxylose [12] (*VIII*, 2.4 g; 7 mmol) was condensed with the disaccharide *VII* (1.3 g; 2.8 mmol) in acetonitrile (12 cm^3) in the presence of mercury(II) cyanide (0.9 g; 3.5 mmol) under the same conditions as described for the foregoing case. After usual working up, the reaction product, *i.e.* the mixture of *XI* and *XII* ($R_f = 0.35$ and 0.3 in the solvent system *C*, twofold development) was isolated by column chromatography on silica gel (550 g, solvent systems $C \rightarrow D$).

The trisaccharide *XI* (yield = 0.45 g (22.5 %)) exhibited m.p. = 160—162 °C after the second crystallization from ethanol and $[\alpha]_D = +9.2°$ ($CHCl_3$). For $C_{30}H_{42}O_{20}$ ($M_r = 722.64$) w_i(calculated): 49.86 % C, 5.86 % H; w_i(found): 49.82 % C, 6.05 % H.

The compound *XII* (yield = 1.35 g (67.5 %)) crystallized from a mixture acetone—ether (volume ratio = 1:2) and after recrystallization showed m.p. = 152—154 °C and $[\alpha]_D = -80.2°$ ($CHCl_3$). Elemental analysis found $w_i = 49.85$ % C and 5.97 % H.

Methyl 3-O-[4-O-(α- and -β-D-xylopyranosyl)-β-D-xylopyranosyl]-β-D-xylopyranosides (XIII and XIV), and methyl 2-O-[4-O-(α- and -β-D-xylopyranosyl)-β-D-xylopyranosyl]-β-D-xylopyranosides (XV and XVI)

The trisaccharide *IX* (0.5 g) was dissolved in a mixture acetone—methanol (volume ratio = 1:5, 20 cm^3). After addition of M methanolic sodium methoxide (1 cm^3), the reaction mixture was kept at laboratory temperature for 2 h. Then t.l.c. showed the absence of the starting *IX* ($R_f = 0.4$ in the solvent system *E*) and the presence of the reaction product *XIII* ($R_f = 0.35$ in the solvent system *F*). The solution was deionized by ion exchange resin Dowex 50-W (H-cycle), filtered and concentrated. The chromatographically pure *XIII* (yield = 0.28 g (94.6 %)) crystallized from ethanol and after recrystallization showed m.p. = 211—212.5 °C and $[\alpha]_D = +18.3°$ (water). For $C_{16}H_{28}O_{13}$ ($M_r = 428.38$) w_i(calculated): 44.86 % C, 6.59 % H; w_i(found): 44.82 % C, 6.66 % H.

The free trisaccharides *XIV*, *XV*, and *XVI* were obtained by the same procedure and in nearly the same yields. The compound *XIV* showed sharp double melting point at 178—180 °C and 199—200 °C (after several crystallizations from methanol, a dimorphic substance) and $[\alpha]_D = -77.4°$ (water). Elemental analysis found $w_i = 44.73$ % C and 6.70 % H.

The compound *XV*, m.p. = 135—138 °C (from methanol), $[\alpha)_D = +13.9°$ (water). Elemental analysis found $w_i = 44.65$ % C and 6.71 % H.

The compound *XVI*, m.p. = 158—159.5 °C (from methanol), $[\alpha]_D = -73.2°$ (water). Elemental analysis found $w_i = 44.62$ % C and 6.73 % H.

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