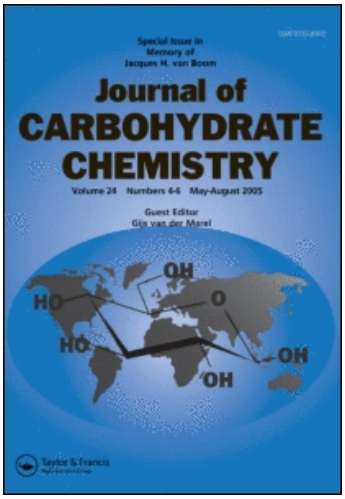

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Syntheses of *P*-Nitrophenyl 6-*O*-α- and 6-*O*-β-D-Xylopyranosyl-β-D-glucopyranoside

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SYNTHESES OF p-NITROPHENYL 6-O-α- and 6-O-β-D-XYLOPYRANOSYL-β-D-GLUCOPYRANOSIDE

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ABSTRACT

Condensation of p-nitrophenyl 2,3,4-tri-O-benzoyl-β-D-glucopyranoside 3 with 2,3,4-tri-O-(chlorosulfonyl)-β-D-xylopyranosyl chloride by the Koenigs-Knorr method afforded the α-linked product in a high yield. Dechlorosulfation with sodium iodide and debenzoylation by the Zemplen method gave crystalline p-nitrophenyl 6-O-(α-D-xylopyranosyl)-β-D-glucopyranoside 7.

Compound 3 was condensed with 2,3,4-tri-O-benzoyl-α-D-xylopyranosyl bromide in the presence of mercury (II) cyanide in acetonitrile, and after debenzoylation, crystalline p-nitrophenyl 6-O-(β-D-xylopyranosyl)-β-D-glucopyranoside 10 was obtained.

INTRODUCTION

Isoprimeverose, 6-O-(α-D-xylopyranosyl)-D-glucose, is a main building unit of the plant xyloglucans which may be firmly associated with cellulose fibrils in the primary cell walls.[1] Many physiological studies have revealed that the xyloglucan plays an important role in the regulation of the cell wall elongation during the cell growth in dicots.[2,3]

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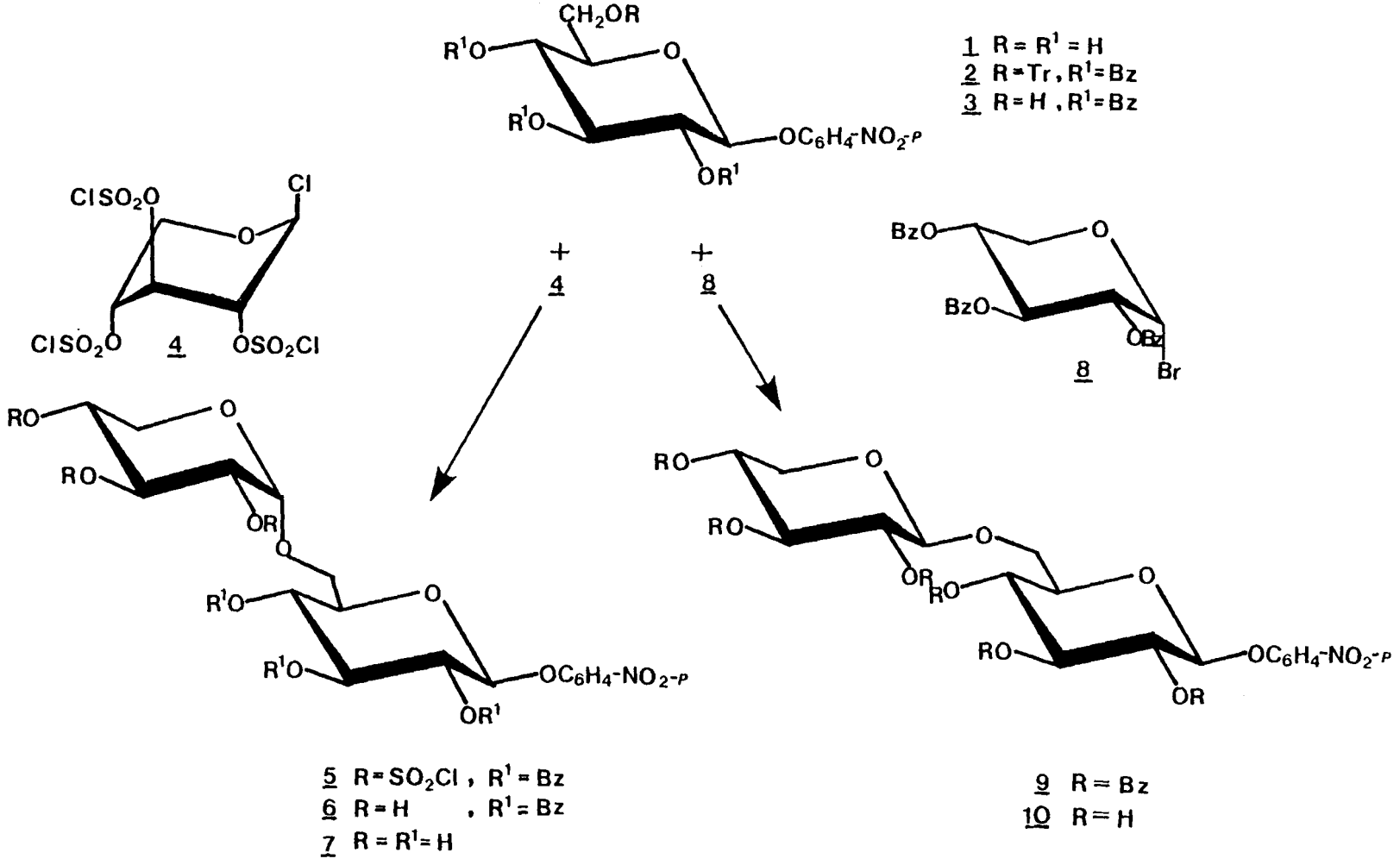
In the course of studies of structure and biosynthesis of plant cell wall polysaccharides, we have prepared the anti-α-L-arabinofuranose antibody by immunization of rabbit with p-azophenyl α-L-arabinofuranoside-bovine serum albumin conjugate. The purified anti-α-L-arabinofuranose antibody was successfully used in the histochemical detection of L-arabinofuranose-containing polysaccharides in the cell wall of various plants.[4]

In this paper, we report the synthesis of p-nitrophenyl 6-O-(α-D-xylopyranosyl)-β-D-glucopyranoside to provide a hapten of the artificial antigen in preparation of the specific antibody to recognize the characteristic building unit of the xyloglucan. We also report the synthesis of p-nitrophenyl 6-O-(β-D-xylopyranosyl)-β-D-glucopyranoside.

RESULTS AND DISCUSSION

The nucleophile, p-nitrophenyl 2,3,4-tri-O-benzoyl-β-D-glucopyranoside (3), was prepared from the commercially available p-nitrophenyl β-D-glucopyranoside (1) by the general method including successive tritylation of the primary hydroxyl, benzoylation of residual hydroxyls, and finally detritylation.

For the synthesis of p-nitrophenyl β-isoprimeverose, as a glycosyl halide, we used 2,3,4-tri-O-(chlorosulfonyl)-β-D-xylopyranosyl chloride (4), which was first introduced to the synthesis of 6-O-α-D-xylopyranosyl-D-mannopyranose.[5] The compound (3) was condenced with the chlorosulfonate (4) in dry chloroform in the presence of $AgCO_3$ and $AgClO_4$ at room temperature. After 24



h the reaction was completed as indicated by the disappearance of the spot corresponding to 3 and appearance of the major spot (R_F =0.54) with a minor spot (R_F = 0.59) on TLC (6:1 toluene-ethyl acetate). An immediate dechlorosulfation of the syrupy condensation product (without the isolation of compound 5) gave an amorphous mass (92% yield based on 3) after evaporation of methanol. The dechlorosulfated product showed only one spot on TLC (R_F = 0.39, 9:1 chloroform-methanol) but its ^{1}H-NMR spectrum in acetone-d_6 showed that it contained p-nitrophenyl 2,3,4-tri-O-benzoyl-6-O-α-D-xylopyranosyl-β-D-glucopyranoside (6) and its β-D-xylopyranosyl anomor in the ratio of 9:1, estimated by the comparison of integration of the α-anomeric proton at 4.89 ppm (doublet, $J_{1,2}$ = 3.66 Hz) and that of the β-anomeric proton at 4.38 ppm (doublet, $J_{1,2}$ = 6.6 Hz). At this step, separation of the α- and β-D-xylopyranosyl products from each other was not successful. The dechlorosulfated product in methanol was treated with sodium methoxide for 2 h, debenzoylation was completed as indicated by the appearance of a main spot (R_F = 0.58) together with a faint spot (R_F = 0.61, found to be identical to that of compound 10) on TLC (3:2:1 ethyl acetate-2-propanol-water). Crystallization from ethanol gave compound 7 whose R_F was identical to that of the main spot of the debenzoylation product in 82% yield. Compound 7 was obtained as needles after recrystallization from water. From the ^{13}C-NMR data of 7, the introduction of the α-D-xylopyranosyl residue at OH-6 in 1 is

confirmed by 5.29 ppm downfield shift of C-6 signal of 7 (to 68.20 ppm from 62.91 ppm of 1) and the chemical shifts of C-1 and C-5 of D-xylopyranosyl residue at 100.23 ppm and 63.53 ppm, respectively.[6,7] Comparison of its optical rotation (-49.3°) to that of 1 (-99.2°) also confirms the α-linkage of D-xylopyranosyl residue.

The compound 3 was condensed with tri-O-benzoylxylopyranosyl bromide (8) in the presence of mercury (II) cyanide in acetonitrile. After overnight reaction, the condensation was completed as indicated by the disappearance of the spot corresponding to 3 and appearance of the new spot between those of 3 and 8 on TLC (10:1 toluene-ethyl acetate). A crystalline condensation product was obtained in high yield (95.5% based on 3) through crystallization from toluene and silica gel chromatography of the mother liquid using 10:1 toluene-ethyl acetate. ^{1}H-NMR and ^{13}C-NMR data were consistent with the structure of 9. The overnight catalytic debenzoylation by the Zemplen method gave syrupy compound 10, in a theoretical yield, which was readily crystallized as needles from 3:2:1 ethyl acetate-2-propanol-water. From the ^{13}C-NMR specrum of 10, β-linked D-xylopyranosyl residue at 6-OH of the starting material (1) is shown by the chemical shifts of C-6 of p-nitrophenyl β-D-glucopyranosyl residue, C-1 and C-5 of D-xylopyranosyl residue at 70.70 ppm, 105.53 ppm and 67.43 ppm, respectively.[6,7] The value of optical rotation of 10, -130.0°, also supports that D-xylopyranose residue is coupled by β-linkage.

For the preparation of artificial antigen, purity and yield of the hapten are very important for several reasons; the immunized animal can produce the antibody to very small amounts of antigen, and we need a quantity of hapten to characterize the antibody specificities. By the simple synthetic procedures described above, the analogues of xyloglucan unit, which can be coupled to an immunogenic protein carrier, could be prepared in high yields (74% for α- and 90% for β- based on starting material 1) and in highly pure crystalline forms.

The preparation and immunological properties of the antibody against the artificial antigens synthesized by couping of BSA and p-animophenyl derivatives of 7 and 10 will be reported elsewhere.

EXPERIMENTAL

General procedures. Melting points were detected with a Yanagimoto micro melting point apparatus without correction. Optical rotations were measured at 23 °C in specified solvents with a Union Giken automatic polarimeter model PM101. Preparative chromatogaphy was performed on columns of slurry-packed Silica Gel 60 (Merck, Cat. No. 9385). Thin-layer chromatography (TLC) was done with pre-coated plates of silica gel (0.25 mm, Merck, Cat. No. 5715) using specified solvent systems. Sugars on TLC plate were detected by charring with 5% (v/v) sulfuric acid in 95% ethanol. ^{1}H NMR (at 400 MHz) and ^{13}C NMR (at 100 MHz) spectra were recorded with a Jeol FMN GX-400 spectrometer. Spectra of 3,

4, 5, 8 and 9 were taken in chloroform-d (internal standard, Me_4Si), that of 6 in acetone-d_6 and those of 1, 7, 10 in D_2O (internal standard, MeOH, MeOH vs. Me_4Si, 49.0 ppm), respectively.

p-Nitrophenyl-2,3,4-tri-O-benzoyl-β-D-glucopyranoside (3). p-Nitrophenyl-β-D-glucopyranoside (1, 2.0 g, 6.6 mmole, Sigma) was dissolved in anhydrous pyridine (26 mL), triphenylmethyl chloride (2.4 g, 8.4 mmole) was added, and the mixture was stirred for 2 days at room temperature, when TLC (85:15 chloroform-methanol) showed no remaining starting material. Benzoyl chloride (2.0 mL, 22 mmole) was added, the solution was kept for 1 h, water (15 mL) was added, and after 30 min, the solution was diluted with dichloromethane, washed with water, and dried (Na_2SO_4). Filtration, evaporation of the solvent, and co-evaporation with toluene, afforded a solid. The solid was dissolved in a mixture of glacial acetic acid (90 mL) and water (10 mL), and the solution was heated at 90 °C for 30 min; when TLC (4:1 toluene-ethyl acetate) showed the reaction to be completed. The mixture was cooled, and evaporated to a syrup. The pure product (2.6 g, 64%) was crystallized from 2-propanol, had mp 121 - 123 °C, $[\alpha]_D$ = + 27.4°, (c 5.0, chloroform); ^{13}C-NMR data (δ): 164.96, 164.27, 164.14, (O-benzoyl, C=O), 160.72 -116.53 (aromatic carbons), 98.34 (C-1), 74.52 (C-5), 72.54 (C-3), 71.39 (C-2), 68.90 (C-4) and 62.22 ppm (C-6).

Anal. Calcd for $C_{33}H_{27}NO_{11}$: C,64.60; H, 4.44; N, 2.28. Found: C, 63.95; H, 4.38; N, 2.18.

p-Nitrophenyl 2,3,4-tri-O-benzoyl-6-O-(α-D-xylopyranosyl)-β-D-glucopyranoside (6)- Compound 3 (1.0 g, 1.6 mmole), silver carbonate (3.0 g, 10.9 mmole), silver perchlorate (0.1 g, 0.5 mmole) and drierite 4A (2.0 g, previously heated at 140 - 160 °C for 60 min and cooled over silica gel) were mixed in 25 ml of dry chloroform (distilled just before the reaction) and stirred vigorously for one hour in an aluminum foil covered flask at 20 - 25 °C. About 2.1 g of crystalline 2,3,4-tri-O-(chlorosulfonyl)-β-D-xylopyranosyl chloride,[5] (4, 4.6 mmole) was then added to the mixture and stirred for additional 24 h. The reaction was monitored by TLC (6:1 toluene-ethyl acetate) and after 24 h the reaction was almost completed as indicated by the disappearance of the spot corresponding to 3 (R_F=0.44). The spot of main product whose R_F was 0.54, appeared between those of 4 (R_F=0.71) and 3 with a very faint spot (R_F=0.61). The reaction mixture was then filtered on a glass filter with an aid of celite layer and the filtrate was concentrated to a syrup. The resulting condensation product (5 with minor its β-anomer) was dissolved in methanol and dechlorosulfated by the addition of a solution of sodium iodide (8 g dissolved in 20 ml of 1:1 methanol-water according to the method described by H. J. Jennings et al.[8] Evaporation of methanol gave a solid (6 with minor its β-anomer, 1.1 g, 92%).

p-Nitrophenyl 6-O-(α-D-xylopyranosyl)-β-D-glucopyranoside (7)- A solution of compound 6 (0.6 g, with a minor β-anomer) in

60 mL of methanol was treated with 3 mL of methanolic 1M sodium methoxide at room temperature until all starting material had disappeared (about 2h), when TLC (3:2:1 ethyl acetate-2-propanol-water) showed that a main product (R_F 0.58) and a minor product (R_F 0.75) were formed. The solution was neutralized with Amberlite IR 120 (H^+-form) resin. The resin was filtered and the filtrate was concentrated to give a solid residue. Crystallization from ethanol gave an amorphous mass of 7 (0.28 g, 80%). Recrystallization of a portion from water gave a needle melting at 240 -241 °C and having $[\alpha]_D$ -49.3° in water (c 1.4) ^{13}C- NMR date (δ): 163.33, 144.61, 128.23 and 118.52 (aromatic carbons); 101.31 (C-1, Glc); 100.19 (C-1, Xyl), 77.86 (C-3, Glc); 77.02 (C-5, Glc); 75.52 (C-3, Xyl); 75.03 (C-2, Glc); 73.66 (C-2, Xyl); 71.68, 71.58 (C-4, Glc, Xyl); 68.20 (C-6, Glc); 63.51 ppm (C-5, Xyl).

Anal. Calcd for $C_{17}H_{23}NO_{12}$: C, 47.12; H, 5.35; N, 3.23. Found; C, 46.29; H, 5.25; N, 3.17.

p-Nitrophenyl 2,3,4-tri-O-benzoyl-6-O-(2,3,4-tri-O-benzoyl-β-D-xylopyranosyl)-β-D-glucopyranoside (9)- The newly prepared 2,3,4-tri-O-benzoyl-α-D-xylopyranosyl bromide,[9] 8, (1.28 g, 2.44 mmole) was added to stirred solution of the nucleophile, 3, (0.5 g, 0.82 mmole) and mercury (II) cyanide (0.65 g, 2.58 mmole) in freshly distilled acetonitrile (10 mL). After overnight stirring at 20 °C, TLC (10:1 toluene-ethyl acetate) showed that only traces of unreacted compound 3 were present. The reaction mixture was diluted with dichloromethane and washed with saturated

solution of $NaHCO_3$ and water. The compound 9 whose R_F was 0.43, was crystallized from toluene (0.50 g). Additional crystals (0.30 g) were obtained after silica gel chromatogaphy of the mother liquid using the same solvent system as that for TLC. The melting point of compound 9 (total yield 800 mg, 95.5 %) was 127 -130 °C and $[\alpha]_D$ in chloroform (c 3.0) was -7.7°. ^{13}C-NMR (δ): 165.22, 165.07, 164.94, 164.82, 164.68, and 164.50 (benzoyl carbons, C=O), 160.91 - 116.42 (aromatic carbons), 100.23 (C-1, Xyl), 98.82 (C-1, Glc), 74.48 (C-5, Glc), 72.57 (C-3, Glc), 71.57 (C-2, Glc), 70.58 (C-6, Glc), 70.30 (C-3, Xyl), 69.28 (C-2, Xyl), 69.22 (C-4, Xyl), 67.68 (C-4, Glc) and 61.62 ppm (C-5, Xyl).

Anal. Calcd for $C_{59}H_{46}NO_{18}$: C, 67.04; H, 4.39; N, 1.33. Found: C, 67.59; H, 4.49; N, 1.35.

p-Nitrophenyl 6-O-(β-D-xylopyranosyl)-β-D-glucopyranoside (10)- A suspension of blocked disaccharide (5, 600 mg) in methanol (30 mL) was treated with 1M sodium methoxide (3 mL). After overnight stirring at room temperature, TLC (10:1 toluene-ethyl acetate) showed that all starting material (R_F 0.43) had disappeared and that a single product was formed (R_F 0.61, 3:2:1 ethyl acetate-2-propanol-water). The solution was neutralized with Amberlite IR 120 (H^+-form) resin and concentrated to give a syrupy material. Crystallization from 3:2:1 ethyl acetate-2-propanol-water gave material (230 mg, 94%) melting at 203 - 204 °C having $[\alpha]_D$ -130° in water (c 0.5). ^{13}C-NMR data (δ): 162.53, 142.65, 128.18, and 118.73 (aromatic carbons), 105.55 (C-1, Xyl),

101.55 (C-1, Glc), 77.94 (C-3, Glc), 77.75 (C-5, Glc), 77.65 (C-3, Xyl), 75.31 (C-2, Xyl), 75.02 (C-2, Glc), 71.59 (C-4, Glc, Xyl), 70.70 (C-6, Glc) and 67.43 ppm (C-5, Xyl).

Anal. Calcd for $C_{17}H_{23}NO_{12}.H_2O$: C, 45.24; H, 5.58; N, 3.10. Found: C, 45.08; H, 5.56; N, 3.07.

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