SYNTHESIS OF POLYSACCHARIDES.

COMMUNICATION 15. SYNTHESIS OF β-(1 → 6)-D-GALACTAN

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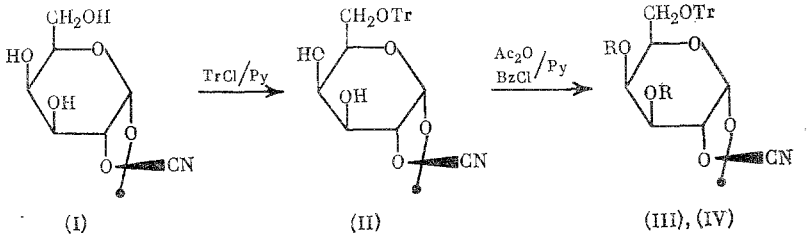
UDC 542.91:547.458

The method of synthesis of homo- and heteropolysaccharides with a regular structure from 6-O-trityl ethers of 1,2-O-cyanoethylidene derivatives of the corresponding mono- and oligosaccharides, developed in the laboratory (review, see [1, 2]), could be used to obtain several polysaccharides with a 1,6-glycoside bond [3-6]. Interest in the study of the immunochemical properties of β-1,6-galactans is related to the production of certain antisera, which are used in the model study of immunoglobulins [7]. Synthetic β-1,6-galactans and their corresponding oligosaccharides are even more important because polysaccharides of this type are rarely encountered in nature. Polysaccharides from *Prototheca zofii* [8, 9] are possibly up to now the only example of nonbranched β-1,6-galactans.

We therefore studied the possible synthesis of β-1,6-galactans by polycondensation of the corresponding cyanoethylidene derivatives. This synthesis is even more interesting because an attempt to obtain β-1,3-galactan unexpectedly led to polysaccharide with disturbed stereoregularity [10] and it was worthwhile to compare this reaction with a polycondensation leading to the formation of 1,6-galactoside bonds.

The initial compound for the synthesis of the required monomers was 1,2-O-[(1-exo-cyano)-ethylidene]-α-D-galactopyranose (I) [10], from which the required 1,2-O-[(1-exo-cyano)ethylidene]-3,4-di-O-acetyl- (III) and 1,2-O-[(1-exo-cyano)ethylidene]-3,4-di-O-benzoyl-6-O-trityl-α-D-galactopyranoses (IV) were obtained according to the scheme given below. In the synthesis of (IV), it was convenient to isolate (II), and then to benzoylate it, otherwise monomer (IV) could be purified on silica gel only with difficulty, and did not crystallize.



(I) (II) (III), (IV)

R = Ac(III), Bz(IV).

Structures (III) and (IV) were confirmed by ^1H and ^{13}C NMR spectra and also by GLC and mass spectra (MS) of acetates of selectively methylated polyols obtained from (III) and (IV) by successive deacetylation, methylation, hydrolysis, reduction with $NaBH_4$, and acetylation. The GLC–MS study of the polyols obtained showed the presence of only 3,4-di-O-methyl-1,2,5,6-tetra-O-acetyldulcite, as confirmed by MS–fragmentation: In the mass spectrum, peaks with m/z: 43, 87, 99, 129, 173, 189, 233 were present.

Compounds (III) and (IV) were polycondensed under the conditions already described in [11]: in dichloromethane, in the presence of 10 mole % of trityl perchlorate as catalyst, at 20°C for 100 h, using a vacuum technique. Comparison of polycondensation products of (III) and (IV) was of special interest, since there are data showing that by using the monomer with a benzoyl protection, a polymer with a higher molecular weight can be obtained [12].

The reaction was stopped by addition of methanol and pyridine (see Experimental), and after the usual treatment and saponification of the polycondensation product according to Zemplen, the polysaccharide fractions were isolated by gel filtration on Sephadex G-25. The yield of the higher molecular weight fractions was about 43%. A study of the structure of

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Translated from Izvestiya Akademii Nauk SSSR, Seriya Khimicheskaya, No. 10, pp. 2358-2363, October, 1984. Original article submitted July 12, 1983.

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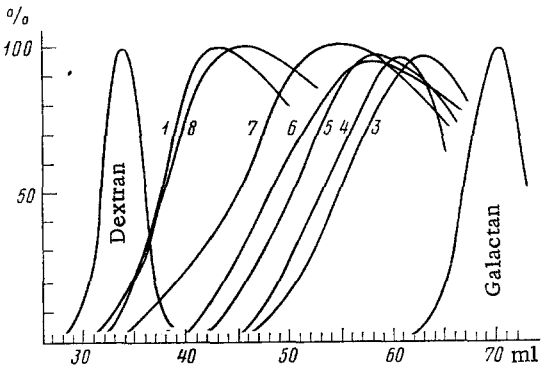

Fig. 1. Gel-filtration curves of polycondensation products of monomer (III): 1, 2) 10 mole % of $TrClO_4$ for 100 h; 3-8) 1 mole % of $TrClO_4$ for 10, 25, 50, 100, 250 h, and 30 days (in coordinates "volume of eluant in ml vs. relative carbon content in fractions," the maximum content was taken as 100%).

the polysaccharide obtained showed that the reaction proceeded with complete regio- and stereospecificity, and GLC–MS analysis of the acetates of the selectively methylated polyols, obtained from the polycondensation products, showed the presence of only 2,3,4-tri-O-methyl-1,5,6-tri-O-acetyl- (V) and 2,3,4,6-tetra-O-methyl-1,5-di-O-acetyl-D-dulcites (VI), indicating the absence of branchings and the presence of 1,6-bonds only. We can thus assume the absence of migration of the different groups in the galactose residue in the polycondensation process. In the ^{13}C NMR spectra of the galactans obtained, in the resonance region of anomeric carbon atoms, only the signal with δ 104.5 ppm was present, which indicates the presence of β-glycoside bonds only in the synthetic polymer obtained (compare with the data on the corresponding tri- and tetrasaccharides [13, 14]), and hence the complete stereospecificity of the polycondensation reaction. The (V):(VI) ratio was about 6 on an average for one or other polycondensation product, and hence the compounds obtained have a low molecular weight (mean degree of polymerization is 7) and are oligosaccharides. Use of the monomer with the benzoyl protection (IV) did not lead to increase in the molecular weight. Nevertheless, the result obtained is interesting from the point of view of the synthesis, if we consider that the step-by-step synthesis of tri- and tetrasaccharides of β-1,6-linked galactopyranose units is labor-consuming [7, 13, 14].

We attempted to increase the molecular weight of the polycondensation product by decreasing the amount of catalyst and varying the time of reaction. A series of preliminary experiments was carried out with 1 mole % of tritylium perchlorate; the duration of the reaction was from 10 h to 30 days. The polycondensation products obtained as the result of reactions carried out for 10, 25, 50, 100, and 250 h were separated on a column with silica gel from all low-molecular-weight products, which in TLC had the mobility of a disaccharide and above (R_f > 0.6, in a benzene:ether = 1:1 system). The residues were subjected to detritylation (90% trifluoroacetic acid), deacetylation and gel chromatography on a column, used for gel filtration of products of the reaction with 10 mole % $TrClO_4$. The product obtained from a reaction carried out for 30 days was treated with CF_3COOH, and then further as in experiments with 10 mole % $TrClO_4$. All the products obtained were deacetylated and gel-chromatographed. The gel filtration curves (Fig. 1) show that the rate of reactions becomes so slow that the degree of polymerization as a function of the time of reaction increases very slowly, and even after 30 days approaches only the degree of polymerization of galactans obtained using 10 mole % of $TrClO_4$. The TLC of the reaction products up to detritylation and subsequent treatment showed that the initial monomer is present in the reaction mixture in a content that decreases with time, although the reaction does not lead a galactan with a higher molecular weight than a reaction with 10 mole % of the catalyst.

The above results show that to obtain polysaccharides with a higher molecular weight by method of polycondensation of cyanoethylidene derivatives, systematic investigation of the reaction kinetics and mechanism are required.

EXPERIMENTAL

The reactions were controlled by means of TLC on silica gel LSL 5/40μ (CSSR). The preparative column chromatography was carried out on silica gel L100/160 (CSSR). The ^1H and ^{13}C NMR spectra were run on the Bruker WM-250 apparatus with TMS or MeOH as internal standards. The optical rotation was measured on the Kofler block. The IR spectra were obtained on the UR-20 apparatus in tablets with KBr. All the solvents were distilled over CaH_2, the catalyst and the monomers were dried directly before the reaction in a vacuum of $3 \cdot 10^{-3}$ mm Hg. The GLC was carried out on the LKhM-8MD chromatograph (3-m steel column, 5% SE-30 on Chromatone N-AW, carrier gas N_2, flame ionization detector). The GLC—MS was carried out on the Varian MAT 111 ("Gnom") apparatus (1.5-m steel column, 3.1 mm in diameter, 5% SE-30 on Chromatone N-AW-DMCS, carrier gas N_2).

1,2-O-[(1-exo-Cyano)ethylidene]-3,4-di-O-acetyl-6-O-trityl-α-D-galactopyranose (III). A 1.59-g portion (5.7 mmoles) of TrCl was added to a solution of 1.29 g (5.6 mmoles) of (I) in 15 ml of absolute pyridine, and after complete dissolution the solution was left to stand for 3 days at about 20°C. The mixture was cooled to 0°C, and 5 ml of Ac_2O were added. It was then left to stand for 2 days at about 20°C, poured into ice water (150 ml), and extracted with chloroform (3 × 50 ml). The extract was washed with ice water (50 ml), saturated solution of $NaHCO_3$ (60 ml), and water (50 ml), then was dried and evaporated to a syrupylike mass (3.1 g). The product was dissolved in a small amount of $CHCl_3$, and precipitated from hexane. The precipitate was crystallized from a mixture of EtOH:MeOH (1:1) to yield 2.06 g (62%) of (III), R_f 0.8 (benzene:ether = 93:7), mp 214°C (MeOH), $[\alpha]_D$ + 28.5° (C 1.0, $CHCl_3$). Found, %: C 69.46, H 5.84, N 2.68. $C_{32}H_{31}O_8N$. Calculated, %: C 68.8, H 5.57, N 2.51. PMR spectrum ($CDCl_3$, δ, ppm) 1.86 s (3H, CH_3C), 1.88 and 2.06 s (6H, CH_3CO), 3.10 d.d (1H, H^6, $J_{6',6} = 9$, $J_{6',5} = 8$ Hz), 3.31 (1H, H^6, $J_{6,6'} = 9$, $J_{6,5} = 5.5$ Hz), 4.20 m (1H, H^5), 4.22 d.d (1H, H^2, $J_{2,3} = 7.0$ Hz), 4.95 d.d (1H, H^3, $J_{3,4} = 2.0$ Hz), 5.56 d.d (1H, H^4, $J_{4,5} = 2.0$ Hz), 5.77 d (1H, H^1, $J_{1,2} = 5.0$ Hz). ^{13}C NMR spectrum ($CDCl_3$, δ, ppm, TMS): 20.30, 26.05, 60.85, 65.80, 71.40, 71.90, 73.00, 87.10, 97.15, 99.25, 117.00, 127.25-143.30, 169.30, and 169.80 ($\underline{C}H_3CO$, $\underline{C}H_3C$, C^6, C^4, C^5, C^3, C^2, $\underline{C}Ph_3$, $\underline{C}CN$, C^1, CN, C_{arom}, $2CH_3CO$).

1,2-O-(1-exo-Cyano)ethylidene-6-O-trityl-α-D-galactopyranose (II). A 615-mg portion (2.2 mmoles) of TrCl was added to a solution of 480 mg (2.1 mmoles) of (I) in 6 ml of pyridine, and the mixture was left to stand for 67 h at about 20°C. A 1-ml portion of MeOH was added and after a few minutes the mixture was evaporated with a toluene—MeOH mixture (1:1). The residue was chromatographed on a column (3 × 20 cm) in a benzene—benzene:ether (1:1) system to yield 720 mg (73%) of a compound, whose individuality was confirmed by TLC (R_f 0.6, benzene:ether = 1:1). By crystallization from a mixture of ethyl acetate—hexane (1:1), 520 mg of (II) were isolated in the form of thin needles, mp 161-163°C, $[\alpha]_D$ +42.1° (C 1.3, $CHCl_3$).

PMR spectrum ($CDCl_3$, TMS, δ, ppm): 1.82 s (3H, CH_3C), 3.40 d.d (1H, H^6, $J_{6,5} = 4.5$, $J_{6,6'} = 11.5$ Hz), 3.50 d.d (1H, H^6, $J_{6',5} = 5.0$, $J_{6',6} = 11.5$ Hz), 3.04 d (1H, 3-OH, $J_{OH,3} = 7.0$ Hz), 3.24 d (1H, 4-OH, $J_{OH,4} = 3.5$ Hz), 3.79 m (1H, H^3), 3.90 m (1H, H^5), 4.01 m (1H, H^4), 4.29 t (1H, H^2, $J_{2,3} = 5.0$ Hz), 5.82 d (1H, H^1, $J_{1,2} = 5.0$ Hz), 7.22-7.44 m (15H, H_{arom}). Found, %: C 70.95; H 5.77; N 2.99. $C_{28}H_{27}O_6N$. Calculated, %: C 71.00; H 5.71; N 2.96.

1,2-O-[(1-exo-Cyano)ethylidene]-3,4-di-O-benzoyl-6-O-trityl-α-D-galactopyranose (IV). A solution of 720 mg (1.52 mmoles) of (II) in 4 ml of pyridine was cooled to 0°C, 0.36 ml (3.2 mmoles) of $BzCl_2$ was added dropwise, and the mixture was left to stand for 68 h at about 20°C. The mixture was poured into 25 ml of saturated solution of $NaHCO_3$ in ice water, extracted with $CHCl_3$ (3 × 15 ml), the extract was washed with water (3 × 10 ml), dried, and evaporated. The residue was chromatographed on a column (2 × 20 cm) in a petroleum ether—petroleum ether:ethyl acetate (4:1) system. Yield, 695 mg of a compound whose individuality was confirmed by TLC (R_f 0.8, 2% ether in benzene). After dissolution in a small amount of $CHCl_3$ and precipitation with MeOH, 685 mg (66%) of (IV) were obtained in the form of a white amorphous powder, mp 88-102°C, $[\alpha]_D$ +55.9 (C2, 2, $CHCl_3$).

PMR spectrum ($CDCl_3$, TMS, δ, ppm): 1.87 s (3H, CH_3C), 3.17 d.d (1H, $H^{6'}$, $J_{6',5} = 9.0$ Hz), 3.38 d.d (1H, H^6, $J_{6,6'} = 9.0$, $J_{6,5} = 6.0$ Hz), 4.32 m (1H, H^5), 4.42 d.d (1H, H^2, $J_{2,3} = 7.0$ Hz) 5.33 d.d (1H, H^3, $J_{3,4} = 3.5$ Hz), 5.92 d.d (1H, H^4, $J_{4,5} = 2.0$ Hz), 5.9 d (1H, H^1, $J_{1,2} = 5.0$ Hz), 7.07-7.80 m (25H, H_{arom}).

^{13}C NMR spectrum ($CDCl_3$, TMS, δ, ppm): 26.0, 61.0, 66.4, 71.6, 72.1, 73.6, 87.2, 97.4, 99.3, 117.0, 127.1-143.3, 165.0, 165.5 ($\underline{C}H_3C$, C^6, C^4, C^5, C^3, C^2, CPh_3, $\underline{C}CN$, C^1, CN, C_{arom}, $Ph\underline{C}O$). Found, %: C 73.75; H 5.26; N 1.96. $C_{42}H_{35}O_8N$. Calculated, %: C 73.8; H 5.14; N 2.06.

Polycondensation of Monomers (III) and (IV). The polycondensation of 560 mg (1 mmole) of (III) and 685 mg (1 mmole) of (IV) was carried out using a vacuum technique [11]: The compounds were dissolved in 5 ml of absolute C_6H_6, and 1-ml portions of this solution were placed in one of the extensions of twin-headed ampuls, while into the other extensions 0.5-ml portions of a solution of 35 mg of $TrClO_4$ in 2.5 ml of absolute $MeNO_2$ were placed. Thus, five reaction ampuls for each monomer were obtained. The solutions of the monomers and the catalyst were lyophilized in a vacuum of $3 \cdot 10^{-4}$ mm, dried for 2 h, and 2 ml of CH_2Cl_2 were introduced into the ampuls by distillation. The contents of the ampuls were mixed to complete dissolution, and the ampuls were left to stand for about 100 h at about 20°C. The ampuls were then opened, 2.0-ml portions of MeOH, and after 30 min, 1-ml portions of pyridine were added to each ampul. The contents of five ampuls of each monomer were combined, and 150 ml of $CHCl_3$ were added to each mixture. The mixtures were washed with H_2O (5 × 25 ml) and evaporated. The residue, after precipitation from a solution in a small amount of $CHCl_3$, was washed with petroleum ether, and dried in air. Yield, 300 mg of polycondensation product 1 from monomer (III) (on TLC plate, band with R_f 0.0-0.2, chloroform–acetone = 9:1) and 415 mg of product from monomer (IV) (R_f 0.0-0.4).

Study of Polycondensation Products. Products 1 and 2 were dissolved in 3 ml of dry $CHCl_3$, 14 ml of absolute MeOH, and 4 ml of 1 N MeONa were added in each case, the mixtures were stirred for 72 h at about 20°C, and evaporated. In the IR spectra of the dry residues, absorption bands at 1750 and 1720 cm^{-1} (the CH_3CO and PhCO groups) were absent. The compounds were dissolved in 2 ml of 0.1 M AcOH, and chromatographed on Sephadex G-25 (a 72 × 1.3-cm column) in 0.1 M AcOH (the total amount of each product was chromatographed in three stages, i.e., $^1/_3$ of the solution was deposited on the column). The rate of elution was 0.4 ml/min. Fractions were collected over a period of 4 min (about 1.5 ml). The content of the carbohydrates was determined by means of the phenol–H_2SO_4 reagent (0.5 ml of 5% solution of phenol in H_2O + 2.5 ml of concentrated H_2SO_4). On the column used, dextran (Dektranblau MG-2000000) comes out with the 28th to 38th ml of the eluent at a maximal concentration in the 34th ml, galactose — from the 64th to 76th ml, maximum concentration in the 70th ml, galactans 1 and 2 begin to come out starting from the 33rd ml, reaching the maximal concentration in the 43rd and 45th ml, respectively. Fractions were collected for galactan 1 up to the 38th ml (23 mg, 13%), and up to the 46th ml (49 mg, 30%), and for galactan 2 — up to the 38th ml (12 mg, 7.5%), and up to the 50th ml (58 mg, 36%). The fractions collected had $[\alpha]_D$ +8.2° (C 1.2, H_2O), +30.8 (C 2.6), +33.0 (C 0.3), and +31.0° (C 1.4). In the ^{13}C NMR spectra of the free galactans (in D_2O, internal standard MeOH, δ 50.15 ppm, with reference to TMS), there is only one signal with δ 104.5 ppm in the resonance region of the anomeric atom C^1, and in the resonance region of other atoms of the galactose units there are signals with δ 75.0 (C^5), 73.9 (C^3), 72.0 (C^2), 70.6 (C^6), 69.9 (C^4), and also low-intensity signals with δ 76.4 (C^5) and δ 62.6 (C^6) of the terminal (from the unreducible end) units, which corresponds to a spectrum of β-(1 → 6)-D-galactan (conforms with ^{13}C NMR spectral characteristics of tri- and tetrasaccharides built up from 1 → 6 linked galactopyranose units [13, 14]). The integral intensities of the latter are related to the intensities of similar signals of units substituted at C^6 in a ratio of 1:6, which corresponds to the mean degree of polymerization of 7.

Methylation and GLC–MS analysis were carried out as already described in [10], using $NaBH_4$ to reduce the hydrolysis products of the methylated samples. Only 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyldulcite (a) and 1,5,6-tri-O-acetyl-2,3,4-tri-O-methyldulcite (b) were detected in a ratio of about 1:6 and identified. The mass spectra of (a) and (b) contained the characteristic peaks, m/z: (a) 71.87, 101, 117, 129, 145, 161, 173, 205; (b) 43, 87, 99, 101, 117, 129, 159, 161, 173, 189, 233.

Polycondensation of Monomer (III) in the Presence of 1 mole % of $TrClO_4$. Portions of 170 mg (0.3 mmoles) of (III) in 1 ml of C_6H_6 were placed into one of the extensions of six twin-headed ampuls, and 0.5-ml portions of a solution of 6.25 mg (0.018 mmoles) of $TrClO_4$ in 3.0 ml of $MeNO_2$ were placed in the other extensions. The solutions were lyophilized and the residues were dried as described above, and then 2-ml portions of CH_2Cl_2 were introduced into the ampuls by distillation, and after complete dissolution the contents of the two extensions were mixed together. The ampuls were left to stand at 20°C, and were opened alternately after 10, 25, 50, 100, 250 h, and 30 days. Mixtures of 1 ml of MeOH and 1 ml of pyridine were added to each ampul, and after 30 min the mixtures were diluted with 30 ml of $CHCl_3$, washed with water and evaporated, and polycondensation products were obtained: 3 (250 mg), 4 (190 mg), 5 (170 mg), 6 (180 mg), 7 (160 mg), and 8 (140 mg) for the above reaction times, respectively. In the TLC of the products obtained, spots appeared with R_f 0.95 (initial

monomer), 0.6, and a band with R_f 0.0-0.2 (benzene:ether = 1:1); the intensity of the spots from the 3rd to the 7th products decreased (in product 8 the spots were practically absent), while the band intensity increased. Products 3-7 were chromatographed on a 1.5 × 20 cm column in a $CHCl_3$ → acetone system, and fractions with R_f < 0.6 were collected. The yield of the products was from 3-35 mg, 4-30, 5-45, 6-50, 7-95 mg; 100, 70, 60, 20, and about 5 mg, respectively, of the initial monomer were recovered. After the above treatment, products 3-8 were dissolved in 1 ml of CF_3COOH (90%), the solutions were held for 1 h, and evaporated with a MeOH—heptane—toluene (1:1:1) mixture to dryness. The residue was additionally dried in an oil pump vacuum, was washed with petroleum ether, and dried again, and was then dissolved in 0.5 ml of absolute $CHCl_3$. Portions of 2 ml of absolute MeOH and 0.8 ml of 1 N MeONa were added, and the mixtures were stirred for 48 h. After evaporation and drying the residues, the absorption bands at 1740 cm^{-1} (C=O) and at 700, 1600, 3030 cm^{-1} (arom. C=H) were absent in the IR spectra of the saponification products. As the result of gel filtration on the above column with Sephadex G-25 it was found that the maximal content of the carbohydrate was, for product 3, in the 63rd ml, 4 — in 60th ml, 5 — in 58th ml, 6 — in 57th, 7 — in 55th, and 8 — in 45th ml of the eluent. The content of the initial monomer in the reaction mixtures after cessation of the reaction decreased from 60% in 3 to 3% in 7, and in mixture 8 the monomer was practically absent.

CONCLUSIONS

1,2-O-(1-exo-Cyano)ethylidene-3,4-di-O-acetyl-6-O-trityl- and 1,2-O-(1-exo-cyano)ethylidene-3,4-di-O-benzoyl-6-O-trityl-α-D-galactopyranoses were synthesized. Their polycondensation led to D-galactans, which do not contain α-glycoside bonds.

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