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SYNTHESIS OF POLYSACCHARIDES.

12.* SYNTHESIS OF REGULAR GLUCORHAMNAN $\rightarrow(\rightarrow 6\text{-}D\text{-Glcp}\,1 \xrightarrow{\beta} 4\text{Rhap}\,1 \xrightarrow{\alpha})_n$

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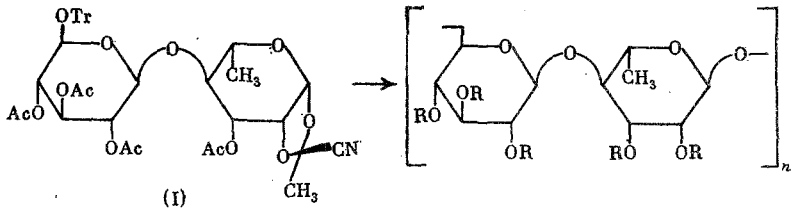
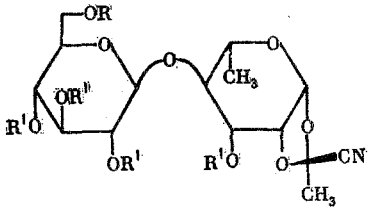
The polycondensation of mono- and oligosaccharide derivatives, containing a 1,2-O-cyanoethylidene group as the glycosylating grouping, and an O-trityl ether as the glycosylated moiety, proceeds both stereo- and regiospecifically, which opened up for the first time a path to the chemical synthesis of polysaccharides, having a regular structure. The use of this path permitted obtaining for the first time a β-1,6-glucan [2], an α-1,3-rhamnan [3], and a glucan containing alternating α-1,4- and β-1,6-glucoside linkages [4]. In this paper we report the synthesis of a regular heteropolysaccharide, and specifically a glucorhamnan that contains the repeating unit $\rightarrow 6\text{-}D\text{-Glcp}1 \xrightarrow{\beta} 4\text{Rhap}1 \xrightarrow{\alpha}$, which was obtained by the polycondensation of 3-O-acetyl-4-O-(2,3,4-tri-O-acetyl-6-O-trityl-β-D-glucopyranosyl)-1,2-O-exo-cyanoethylidene-β-L-rhamnopyranose (I).



(I)

To obtain (I) the full acetate of the 1,2-O-exo-cyanoethylidene derivative of β-D-glucopyranosyl-(1→4)-β-L-rhamnopyranose (II) [5] was deacetylated by treatment with CH_3ONa/CH_3OH, and the obtained derivative (III), without isolation, was treated with triphenylchloromethane in pyridine. The tritylation product (IV) was acetylated and, after chromatography on SiO_2, the crystalline (I) was isolated in 49% yield [based on (II)].



R=Ph_3C; R′=Ac (I); R=R′=Ac (II); R=R′=H (III); R=Ph_3C, R′=H (IV).

The structure of (I) was confirmed by the PMR spectrum, which contains the following signals: the CCH_3 of the 1,2-cyanoethylidene grouping (δ 1.7 ppm), the $CHCH_3$ group of rhamnose (δ 1.43 ppm), the H^1 of rhamnose (δ 5.38 ppm, $j_{1,2} = 2$ Hz), and also the signals of four O-acetyl groups (δ 1.86-2.22 ppm) and 15 aromatic protons (δ 7.2-7.7 ppm).

The polycondensation of monomer (I) was run in CH_2Cl_2 in the presence of 0.1 mole of tritylium perchlorate for 50 h, under conditions close to those described previously [2, 4]. Increasing the reaction time to 250 h failed to give a polymer with a higher molecular weight, which was established by the fact that the analytical gel-filtration curves of the polycondensation products, after removal of the the protective groups, on Biogel P-10 coincided. The reaction was stopped by the successive addition of methanol and pyridine, and the

*See [1] for Communication 11.

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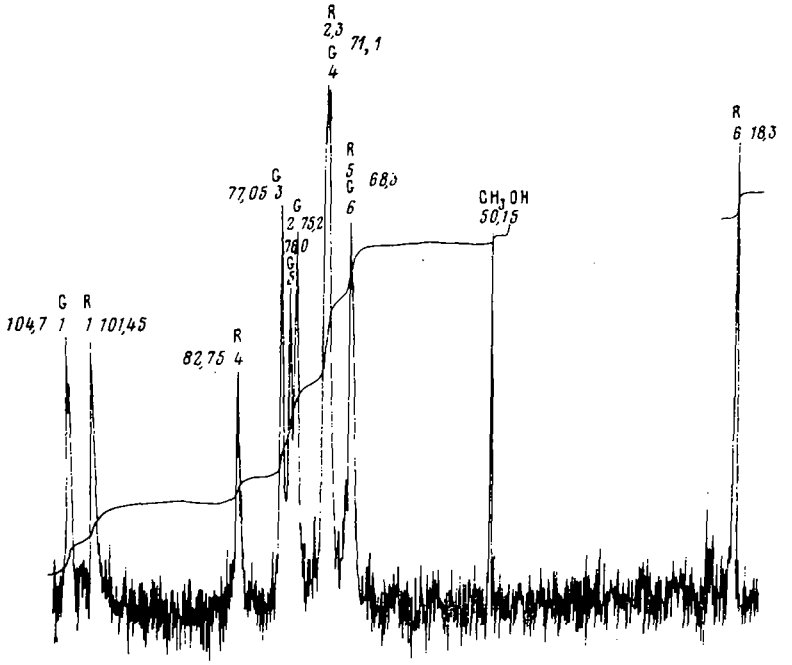

Fig. 1. ^{13}C NMR spectrum of glucorhamnan.

obtained polymer was deacetylated with CH_3ONa/CH_3OH, checking the completeness of deacetylation via IR spectroscopy.

The analytical gel-chromatography of the obtained polysaccharide on Sephadex G-25 and Biogel P-10 disclosed that the product is devoid of lower oligosaccharides. The obtained polysaccharide was separated into two fractions (yields of 38.9 and 47.8%), which differed in the degree of polymerization, by preparative gel-chromatography on Biogel P-10.

Determination of the monosaccharide composition of the obtained polysaccharide as the polyol acetates by the GLC and GLC-MS method disclosed that both fractions give only sorbitol and rhamnitol in a 0.93:1 ratio, which testifies to the absence of any transformations of the starting monomer during polycondensation. Analysis of both polysaccharide fractions via methylation and subsequent identification of the partially methylated polyol acetates by the GLC-MS method disclosed the presence of only 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-sorbitol (V), 1,5,6-tri-O-acetyl-2,3,4-tri-O-methyl-D-sorbitol (VI), and 1,4,5-tri-O-acetyl-2,3-di-O-methyl-L-rhamnitol (VII). The obtained data testify to the strict regiospecificity of the accomplished polycondensation and to the strict regularity of the obtained polysaccharide. The ratio of the tetramethyl-substituted sorbitol (V) to the trisubstituted sorbitol (VI) is ∿1:29 for fraction I, and ∿1:22 for fraction II, which corresponds to an average degree of polymerization of the disaccharide units of 30 and 23, and average molecular weights of ∿9000 and 7000, respectively.

It is important to emphasize that the methylation products failed to contain even traces of 1,5-di-O-acetyl-2,3,4-tri-O-methyl-L-rhamnitol. This testifies to the fact that cleavage of the glycoside linkages in either the starting monomer (I) or the already formed polysaccharide chain fails to occur during the polycondensation process.

The stereoregularity of the obtained polymer follows from the ^{13}C NMR spectra of the mixture of fractions I and II (see Fig. 1). The absence of signals at 74.0 (C^3) and 72.8 ppm (C^5) of the β-rhamnopyranose moiety in the spectrum testifies [6-8] to the complete absence of β-rhamnoside linkages in the polymeric chains. The cleanness of the resonance region of the anomeric C atoms in the spectrum of the polymer (two peaks with δ 101.45 (C^1 of rhamnopyranose moiety) and 104.7 ppm (C^1 of glucopyranose moiety)) also confirms the regularity of the polysaccharide. A complete deciphering of the ^{13}C NMR spectra (Table 1). of the polysaccharide is obvious from a comparison of the spectra of the polymer and model saccharides.

$$L\text{-Rhap1} \xrightarrow{\alpha} 6\text{-}\beta\text{-}D\text{-Glcp} \quad \text{(VIII) [6]}$$

TABLE 1. ^{13}C NMR Spectra Data for Polysaccharide and Model Compounds (D_2O, internal standard = CH_3OH, 50°C)

Compound	Chemical shifts of rhamnose moiety, ppm						Chemical shifts of glucose moiety, ppm					
	C^1	C^2	C^3	C^4	C^5	C^6	C^1	C^2	C^3	C^4	C^6	C^6
Polysaccharide	101,45	71,1	71,1	82,75	68,3	18,3	104,7	75,2	77,05	71,1	76,0	68,3
(VIII)	101,9	71,4	71,7	73,5	69,9	17,9	97,4	75,5	77,2	71,2	76,1	68,3
(IX)	102,5	71,4	71,8	82,5	68,25	18,1	104,7	75,2	77,3	71,0	77,3	62,1
(X)	94,5	71,8	74,0	80,4	72,8	18,3						

D-Glcp1 $\xrightarrow{\beta}$ 4-*L*-Rhap1 $\xrightarrow{\alpha}$ OMe (IX) [7]

D-Manp1 $\xrightarrow{\beta}$ 4-β-*L*-Rhap (X) [8]

The obtained glucorhamnan has a quite high molecular weight and in its degree of polymerization is close to that of the α-1,3-rhamnan described in a previous communication [1]. The previously described synthetic glucans [2, 4] were obtained under the same conditions, but with a lower degree of polymerization. This difference must apparently be attributed to the higher reactivity of the 1,2-O-cyanoethylidene derivative of rhamnose when compared with the analogous glucose derivatives.

EXPERIMENTAL

The TLC was run in a loose layer of SiO_2, the solvents were prepared as described in [3], and the GLC was run on an LKhM 8MD instrument (3-m long steel columns packed with 5% SE-30 deposited on Chromaton N-AW). The PMR spectra were taken on a Tesla BS-497 instrument (100 MHz) relative to TMS. The GLC-MS was run on a Varian MAT Gnom-111 instrument (1.5-m long steel columns packed with 3% SE-30 deposited on Varoport-30). The specific rotation was determined on a Perkin—Elmer 141 polarimeter, and the melting points were determined on a Kofler block. The ^{13}C NMR spectrum of the polysaccharide was taken on a Bruker WP-60 instrument (15.08 MHz, D_2O, internal standard = CH_3OH, 50.15 ppm relative to TMS) at 50° for a 5% solution.

3-O-Acetyl-4-O-(2,3,4-tri-O-acetyl-6-O-trityl-β-D-glucopyranosyl)-1,2-O-exo-cyanoethylidene-β-L-rhamnopyranose (I). To a solution of 1.4 g (2.4 mmoles) of 3-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-1,2-exo-cyanoethylidene-β-L-rhamnopyranose (II) [5] in 3.6 ml of dry $CHCl_3$ was added 7.2 ml of a 0.005 M MeONa solution in abs. MeOH and the mixture was let stand for 30 min at ∿20°. Then 0.48 ml of a 0.1 M AcOH solution in abs. MeOH was added and the mixture was evaporated to dryness. The residue (0.7 g) was dried in vacuo, dissolved in 12 ml of abs. pyridine, 1.2 g (4.4 mmoles) of triphenylchloromethane was added, and the mixture was let stand at ∿20° for 3 days. Then 5 ml of Ac_2O was added and the mixture was let stand for another 15 h. To the mixture was then added 5 ml of MeOH and the mixture was repeatedly evaporated, first with methanol and then with toluene. The solid residue was washed repeatedly with petroleum ether, chromatographed on SiO_2 (benzene—ether), and recrystallized from alcohol. We obtained 0.90 g (49%) of (I), mp 256°, $[\alpha]_D^{20}$ +15.4° (C 2.2, $CHCl_3$). PMR spectrum (δ ppm): 1.43 d (CH_3CH of rhamnose), 1.7 s (CH_3C), 1.86, 1.96, 2.0, 2.22 (four OAc), 5.38 (H^1 of rhamnose, $J_{1,2}$ = 2 Hz), 7.2-7.7 (15 H, aromatic protons). Found: C 64.08; H 5.86; N 1.92%. $C_{42}H_{45}O_{14}N$. Calculated: C 64.03; H 5.76; N 1.78%.

Polycondensation of Monomer (I). The polycondensation was run using the high-vacuum technique described in [2-4]. In the prongs of a two-prong ampul were placed solutions of 330 mg (420 μmoles of (I) in abs. benzene and 14.4 mg (42 μmoles) of tritylium perchlorate in abs. nitromethane. The solutions were frozen and lyophilized, after which 3 ml of abs. CH_2Cl_2 was distilled into the ampul, and the prong contents were mixed and let stand for 50 h at ∿20°. The reaction was stopped by adding excess abs. MeOH and pyridine. To the mixture was then added 15 ml of $CHCl_3$, the mixture was washed with water (5 × 15 ml), and the $CHCl_3$ was evaporated.

Deacetylation of Polycondensation Product and Its Study by Gel-Chromatography Method. To a solution of the polycondensation product in 15 ml of abs. MeOH was added 3 ml of a 1 N MeONa solution in abs. MeOH, after which the mixture was stirred for 72 h and then evaporated

to dryness. The residue was dissolved in 10 ml of 0.1 N AcOH solution, and 0.3 ml of this solution was chromatographed on Sephadex G-25 and Biogel P-10 in 0.1 N AcOH solution (59 × 1.1 cm column). The amount of sugars in the fractions was determined using the phenol—H_2SO_4 reagent. On Sephadex G-25 the polysaccharide exits with the eluant front, while on Biogel P-10 it begins to exit with the eluant front, but the main fraction exits in half of the entire column volume. The polysaccharide was subjected to preparative gel-chromatography on a column (90 × 1.4 cm) packed with Biogel P-10 in 0.1 N AcOH solution. Two fractions of the polysaccharide were collected: fraction I (from 34 to 54 ml of the eluate), 53 mg (38.9%), and fraction II (from 54 to 78 ml of the eluate), 65 mg (47.8%), which were lyophilized and dried in vacuo at 100°.

The mixture of polysaccharides I and II has $[\alpha]_D^{20}$ −52.5° (C 1.1, water). Cf. $[\alpha]_D$ −57.5° (CH_3OH) for β-D-Glcp-(1→4)-α-L-Rhap-OMe [9].

Determination of Structure of Polysaccharides of Fractions I and II. For this 5 mg each of the polysaccharide of fractions I and II was subjected to formolysis (2 ml of 85% HCOOH, 100°, 2 h), hydrolysis (2 ml of 0.1 N HCl solution, 100°, 15 h), reduction (2 ml of water, 50 mg of $NaBH_4$, 20°, 15 h), and acetylation (1 ml of pyridine, 1 ml of Ac_2O, 20°, 15 h). In both cases it was shown that only 1,2,3,4,5-penta-O-acetyl-L-rhamnitol and 1,2,3,4,5,6-hexa-O-acetyl-D-sorbitol are present in a 0.93:1 ratio, which were identified by the GLC and GLC-MS method.

Methylation of Glucorhamman. Here 5 mg each of the polysaccharide of fractions I and II was dissolved in 1 ml of abs. DMSO and added to a solution of dimsylsodium (from 25 mg of NaH and 1.5 ml of abs. DMSO), after which the mixture was let stand for 5 h and then 5 ml of MeI was added. After 24 h 10 ml of $CHCl_3$ was added, and the solution was washed with water (8 × 10 ml) and evaporated. The methylated polysaccharides were subjected to formolysis, hydrolysis, reduction, and acetylation as described above, and then studied by the GLC and GLC-MS method. Here we identified only (V) (mass spectrum, m/e: 71, 87, 101, 113, 117, 129, 145, 161, 205), (VI) (mass spectrum, m/e: 71, 74, 75, 101, 117, 129, 159, 162, 173, 189, 233), and (VII) (mass spectrum, m/e: 87, 101, 117, 129, 143, 161, 203), in which connection the ratio of (V) to (VI) for the polysaccharide of fraction I was ∿1:29, and ∿1:22 for the polysaccharide of fraction II.

CONCLUSIONS

We synthesized the monomer, 3-O-acetyl-4-O-(2,3,4-tri-O-acetyl-6-O-trityl-β-D-glucopyranosyl)-1,2-O-exo-cyanoethylidene-β-L-rhamnopyranose, and accomplished its polycondensation, as a result of which a stereoregular glucorhamnan was obtained.

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