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THE EFFECT OF HIGH PRESSURE ON THE STEREOSPECIFICITY OF THE GLYCOSYLATION REACTION*

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ABSTRACT

At a pressure of 1.4 GPa and room temperature, the glycosylation of trityl ethers by 1,2-*O*-cyanoethylidene derivatives of sugars, and the polycondensation of the proper monomers in dichloromethane, proceed with absolute stereospecificity, giving rise to a 1,2-*trans*-glycosidic linkage, although, at the ambient pressure, the reactions studied reveal a rather low stereospecificity. The effect of high pressure on the stereospecificity of glycosidic linkage formation is explained as being due to the shift of the equilibrium between monocyclic glycosyl-cation and bicyclic acyloxonium cation towards the latter as the pressure is increased.

INTRODUCTION

The glycosidic linkage is practically the only linkage between sugar units in natural, carbohydrate-containing biopolymers and their fragments. In this connection, the methods of glycosylation, which were developed in work by Fischer, Koenigs and Knorr, and Helferich, whose centenary is being marked this year, are the key synthetic methods in carbohydrate chemistry. The most intricate problem of the glycosylation reaction is that of its stereospecificity, which, until the present, has not been resolved on general grounds, thus hampering progress in synthetic work. There have been numerous investigations devoted to the study of the influence of the nature of promoters, the solvents, the temperature, and the structure of the aglycon component being glycosylated on the stereospecificity of glycosylation. However, the influence of pressure on the progress of this reaction, in particular on its stereospecificity, has not been studied to date.

During recent decades, study of the influence of high pressure (100 MPa–2.0 GPa) on the progress of a series of chemical reactions, and its utilization for synthetic purposes, have attracted considerable attention (*cf.* review paper[1]), but still the stereochemical aspects of the action of high pressure have been studied quite poorly,

*Dedicated to the memory of Professor Burckhardt Helferich on the hundredth anniversary of his birth

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and the influence of pressure in the reactions studied was, as a rule, insignificant (*cf.* refs. 2 and 3).

In the present work, we report on the striking influence of high pressure on the reaction of glycosylation of trityl ethers with 1,2-*O*-cyanoethylidene derivatives of sugars. This reaction is known (see review papers, refs. 4 and 5) to be employed for the preparation of oligosaccharides, and was the basis for the first general method for the synthesis of polysaccharides. In most cases, the reaction proceeds with absolute stereospecificity, leading to the exclusive formation of the 1,2-*trans*-glycosidic linkage. However, it has recently been recognized that, in the case of 3- and 4-*O*-trityl derivatives of glucose, galactose, xylose, and arabinose, the stereospecificity is violated[6-9]. With a view to improving the stereospecificity and extending the scope of the stereospecific synthesis of polysaccharides, we have studied the influence of high pressure on the reaction of glycosylation of the trityl ethers with 1,2-*O*-cyanoethylidene derivatives of sugars. It was established that, under a pressure of 1.4 GPa (14 kbar), the reaction is shifted to the formation of the 1,2-*trans*-glycosides, and the glycosylation proceeds with absolute stereospecificity in cases in which, under normal pressure, stereospecificity is violated.

RESULTS AND DISCUSSION

In order to elucidate the influence of high pressure on the stereospecificity of glycosidic-linkage formation upon interaction between the 1,2-*O*-cyanoethylidene derivatives of sugars and trityl ethers of sugars (the trityl–cyanoethylidene condensation), there were chosen particular examples of syntheses of the simplest oligosaccharides and homopolysaccharides, in which this stereospecificity is violated the most strongly under the usual conditions.

SYNTHESIS OF DISACCHARIDES

For the study of the "unfavorable" syntheses of disaccharides, we chose the reactions between 3,4,6-tri-*O*-acetyl-1,2-*O*-[1-(*exo*-cyano)ethylidene]-α-D-glucopyranose (**1**) and 1,2,4,6-tetra-*O*-acetyl-3-*O*-trityl-β-D-glucopyranose (**2**) and methyl 3,4,6-tri-*O*-acetyl-2-*O*-trityl-α-D-glucopyranoside (**3**), as well as between 3,4,6-tri-*O*-acetyl-1,2-*O*-[1-(*exo*-cyano)ethylidene]-α-D-galactopyranose (**4**) and methyl 2,3-di-*O*-acetyl-4-*O*-trityl-β-D-xylopyranoside (**5**) and 3,4-di-*O*-acetyl-1,2-*O*-[1-(*exo*-cyano)ethylidene]-6-deoxy-α-D-glucopyranose (**6**) with **2**.

The reaction was carried out in the presence of 7–10 mol% of tritylium perchlorate in dichloromethane solution. In all cases, the control experiments were carried out *in vacuo* under the standard conditions of the trityl–cyanoethylidene condensation described previously[6,7]. The experiments under pressure were performed in a hermetically sealed Teflon ampoule placed in a reaction vessel under a pressure of 1.4 GPa. When the reaction was to be terminated, the pressure was released, the reaction mixture was diluted with chloroform, and CH_3OH–C_5H_5N

TABLE I

SYNTHESIS OF DISACCHARIDES BY THE CYANOETHYLIDENE METHOD

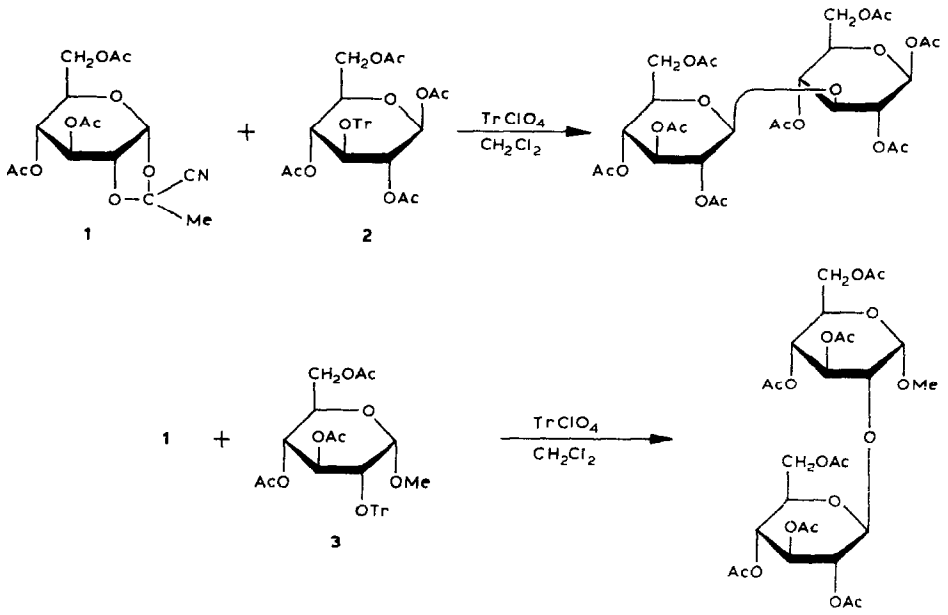
Glycosylating agent	*Aglycon*	*Reaction conditions*			*Total yield %*	*1,2-trans: 1,2-cis ratio*
		Pressure	*$TrClO_4$ (mol %)*	*Time (h)*		
1	2	0.1 MPa	10	24	57	59/41
1	2	1.4 GPa	10	3	87	100/0
1	3	0.1 MPa	7	60	64	71/29
1	3	1.4 GPa	7	24	79	97/3
4	5	0.1 MPa	10	15		70/30
4	5	1.4 GPa	10	2.5		77/23
4	5	1.4 GPa	5	2.5		100/0[a]
6	2	0.1 MPa	4	20	68	34/66
6	2	1.4 GPa	4	2.5	90	100/0[b]

[a]After mixing the reagents, and prior to application of pressure the ampoule was cooled to −75°.
[b]After mixing the reagents, and prior to application of pressure the ampoule was cooled to −196°.

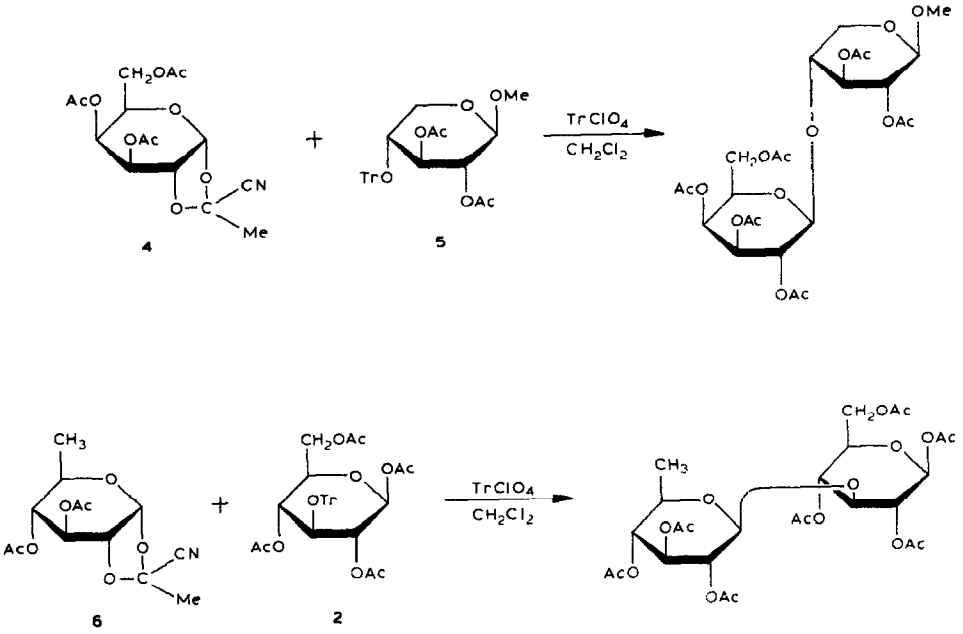
was added. The mixture of disaccharides formed was isolated by chromatography, and the ratio of 1,2-*trans*- to 1,2-*cis*-linked disaccharides was determined by comparing the integrated intensities of the signals of anomeric carbon atoms belonging to the non-reducing unit. The results of the experiments are listed in Table I.

The first experiments showed that the pressure shifts the ratio of 1,2-*trans*- to 1,2-*cis*-disaccharides (*i.e.*, the ratio of β/α anomers) towards formation of the *trans* isomer, so that, in some cases, only the β-anomer is formed. However, in other cases, the formation of the *cis* isomer was often observed, and, thus, absolute stereospecificity of glycosylation was not attained. Thus, for example, condensation of **1** and **2** under the usual conditions yields a β/α ratio of 59:41 (total yield, 57%), whereas the reaction performed under a pressure of 1.4 GPa yields only the β anomer, namely *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→3)-1,2,4,6-tetra-*O*-acetyl-β-D-glucopyranose, in 87% yield. On the other hand, the interaction between **4** and **5** under the usual conditions affords a mixture of disaccharides with a β/α ratio of 70/30, whereas, under a pressure of 1.4 GPa, this ratio is changed to 77/23. It was suggested that, in those cases where the formation of the 1,2-*cis* isomer (α anomer) nevertheless takes place, this is because the reaction of glycosylation of a trityl ether occurs so rapidly that it partially proceeds before the working pressure is reached, and the reaction occurring under these conditions results in low stereospecificity. A control experiment between **4** and **5** showed that, during the period between mixing the reagents and achieving a pressure of 1.4 GPa (~30 min), the mixture produces a considerable amount of the disaccharides.

In order to avoid this misleading phenomenon, the ampoule with the mixed reagents was immediately cooled to −75 or −196°, and the cooled ampoule was placed in the reaction vessel at room temperature (20°), in which a pressure of 1.4 GPa was reached in 8 min. Actually, in these cases, the reaction at low temperature



was completely hampered, and was only initiated at high pressure. The data in Table I show that only the 1,2-*trans* isomer, *viz.*, methyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3-di-*O*-acetyl-β-D-xylopyranoside was formed in this case, *i.e.*, the reaction proceeded with absolute stereospecificity.



Although some influence of the temperature change on the reaction course cannot be completely neglected (an ampoule might not acquire room temperature right away), the results obtained show that high pressure plays a decisive role in shifting the reaction toward the formation of the 1,2-*trans*-glycosidic linkage. This effect was observed for the cyanoethylidene derivatives of different structures (derivatives of glucose, galactose, and 6-deoxyglucose) and various trityl ethers involving a trityl group at O-2,-3 and -4 of hexo- and pento-pyranoses. Therefore, the effect of high pressure appears to be of a sufficiently general nature to allow performing the synthesis of oligosaccharides with absolute stereospecificity.

The striking effect of high pressure on the stereochemistry of glycosylation deserves especial attention, because it permits acquisition of a deeper insight into the mechanism of this reaction. In addition to the data listed, in one of the reactions of disaccharide synthesis (between **1** and **3**), where no preliminary cooling of the starting reaction mixture was required, the β/α ratio was determined at several intermediate pressures ranging from 0.1 MPa to 1.4 GPa, giving, as a result, a smooth dependence of log (β/α) on pressure which is presented in Fig. 1. This relates the effect of pressure on the particular reaction to the peculiarities of its mechanism, and not to a possible phase-transition of the solvent, dichloromethane, in the region of 12–14 GPa, which could affect the stereoselectivity of the process.

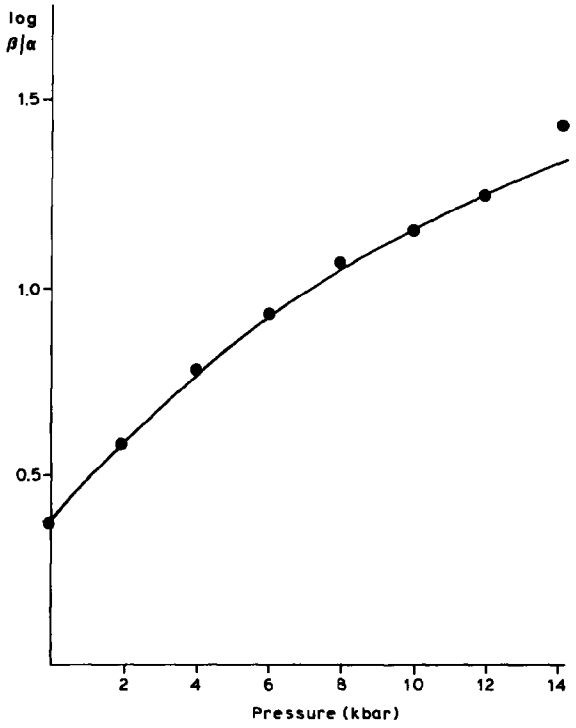


Fig. 1. Dependence of log (β/α) on pressure for the reaction of **1** with **3**.

On the basis of the data, both those obtained by us and those available in the literature, on the influence of pressure on chemical reactions, the following mechanism of formation of β and α anomers may be suggested. In the process proceeding stereoselectively at atmospheric pressure, the β anomer is obtained, due to the formation, as an intermediate, of a bicyclic acyloxonium cation upon abstraction of a cyano group from the cyanoethylidene derivative under the action of the trityl cation, and the rearrangement of this cation into a monocyclic cation practically does not occur[5]. In the non-stereoselective process, the glycosyl cation is, in fact, formed and its interaction with the oxygen atom of the trityloxy group of the aglycon yields both the β and the α anomer. If this is the case, the effect of high pressure on the β/α ratio is effectively reduced to one of changing an equilibrium between the bicyclic and monocyclic cations. With growing pressure, the equilibrium is shifted towards the formation of the bicyclic cation, because cyclization is usually accompanied by a noticeable decrease in volume. As a consequence, at a sufficiently high pressure, the concentration of the monocyclic glycosyl cation becomes so low that there occurs the formation of only the β anomer. This working hypothesis needs careful verification.

SYNTHESIS OF POLYSACCHARIDES

As already pointed out, study of the effect of high pressure on the stereochemistry of the trityl–cyanoethylidene condensation was carried out in the first place with a view to extending the scope of a method for the synthesis of polysaccharides that is based on this reaction. Therefore, the results obtained were employed in the polycondensation of monomers, having both an *O*-trityl group and a cyanoethylidene moiety on the same monosaccharide units, in the presence of tritylium perchlorate, with the formation of polysaccharide chains (*cf.* review papers[4,5]). Although, in many cases, these reactions proceed with absolute stereospecificity, in some cases, the stereospecificity is violated, to give stereochemically irregular polysaccharide.

In addition, although in some cases the reaction is stereospecific, the degree of polymerization attained upon polycondensation is extremely low, and the reaction affords not a polysaccharide, but rather a mixture of higher oligosaccharides in quite a poor yield. This takes place, *e.g.*, in the synthesis of (1→6)-β-D-galactan[10].

It might be supposed that performing the polycondensation at high pressure would elevate both the stereochemical specificity of the reaction and the degree of polymerization of the polymer formed. With a view to verifying this suggestion, we carried out the synthesis of three polysaccharides which, upon polycondensation of the components under the usual conditions, afforded a particularly unsatisfactory result, namely, the synthesis of (1→3)-6-deoxy-β-D-glucan and (1→3)-β-D-galactan, which involve a large number of "anomalous" α linkages, and (1→6)-β-D-galactan.

For the synthesis of (1→3)-6-deoxy-β-D-glucan, we studied the polyconden-

sation of 4-*O*-acetyl-1,2-*O*-[1-(*exo*-cyano)ethylidene]-6-deoxy-3-*O*-trityl-α-D-glucopyranose (**10**).

For the synthesis of the monomer **10**, compound **7** (obtained formerly[6]) was deacetylated with methanolic sodium methoxide in pyridine; free 1,2-*O*-[1-(*exo*-cyano)ethylidene]-6-deoxy-α-D-glucopyranose (**8**) was, by treatment with tritylium perchlorate, converted into the 3-*O*-trityl derivative **9**, which, on acetylation, gave the monomer **10**. Polycondensation of **10** at atmospheric pressure under the glycosylation conditions specified previously[6], afforded, after standard workup of the reaction mixture followed by deacetylation, the polysaccharide (yield 63%). Methylation analysis showed the presence of only the (1→3)-glycosidic linkage, and the average degree of polymerization was 15. According to ^{13}C-n.m.r.-spectral data, the polysaccharide had 41% of β-glycosidic linkages (the signals in the region of 102–104 p.p.m.), and 59% of α-glycosidic linkages (the signals in the region of 98–100 p.p.m.). In the region where the ring-carbon atoms resonate, the spectrum contained a large number of hardly interpretable signals, which indicated an irregular distribution of α and β linkages along the polysaccharide chain (see Fig. 2b). Thus, the polycondensation at atmospheric pressure afforded a (stereochemically) fully irregular polysaccharide.

In this connection, the polycondensation of monomer **10** was studied under the same conditions except for pressure, which was set at 1.4 GPa. After standard

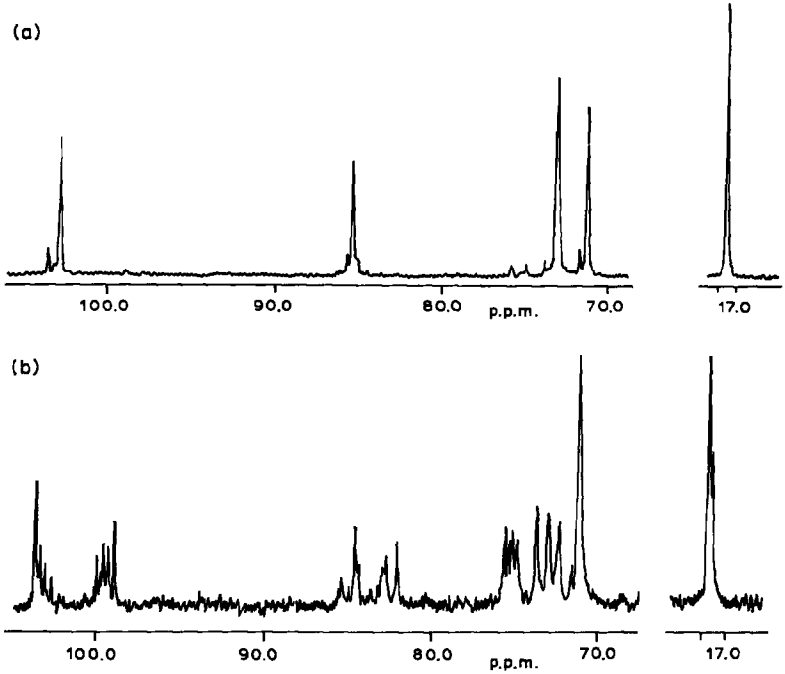
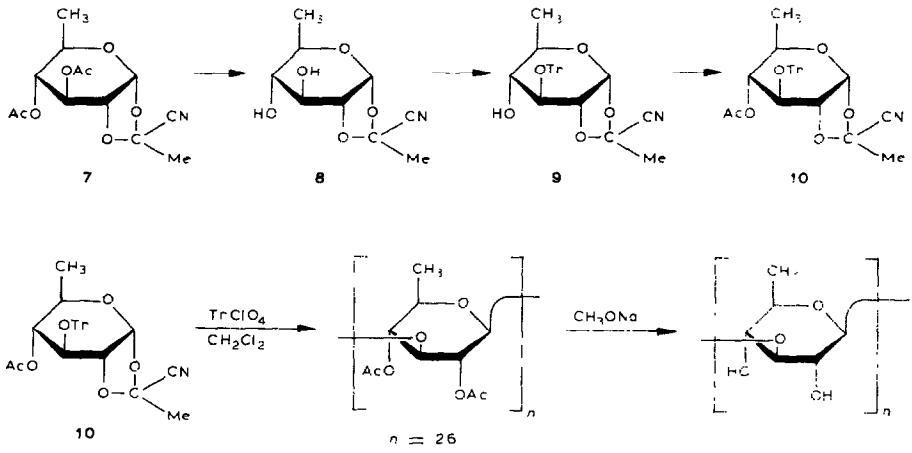


Fig. 2. (a) ^{13}C-n.m.r. spectrum of (1→3)-6-deoxy-β-D-glucan obtained under a pressure of 1.4 GPa; (b) ^{13}C-n.m.r. spectrum of (1→3)-6-deoxy-D-glucan obtained under atmospheric pressure.

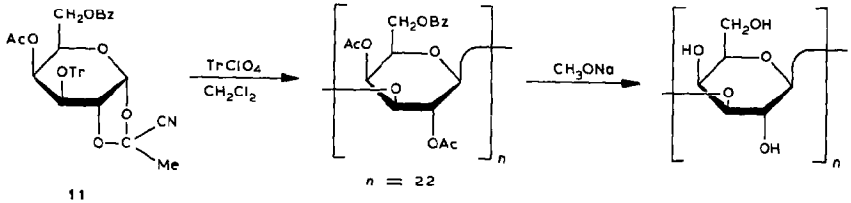
work-up of the reaction mixture, the ^{13}C-n.m.r. spectrum of the polymer acetate thus obtained showed the ratio of the β-glycosidic to the α-glycosidic linkages to be 56/44. Therefore, as in the case of the disaccharide synthesis, the high pressure caused the predominant formation of β-glycosidic linkages. With a view to avoiding occurrence of any polycondensation prior to the application of high pressure, and taking into account the data on the synthesis of disaccharides, further experiments were conducted with smaller proportions of tritylium perchlorate (up to 4 mol%), using a more dilute solution of the monomer, cooling the ampoules containing the reaction mixture to −196°, and cooling the high-pressure vessel to 0° prior to application of high pressure. The ampoule was placed in the vessel, the cooling of the vessel was terminated, and the pressure was elevated to 1.4 GPa in 8 min and maintained there for 20 h. Control experiments showed that the vessel acquires room temperature in 1.5 h.



Because the polysaccharide acetate proved to be insoluble in methanol–chloroform (thus proving a highly regular structure and relatively high degree of polymerization), its deacetylation was performed with methanolic sodium methoxide in Me_2SO. Methylation analysis showed the presence of (1→3)-glycosidic linkages exclusively, and an average degree of polymerization of 26. The ^{13}C-n.m.r. spectrum of the (1→3)-6-deoxy-β-D-glucan obtained (see Fig. 2a) contained only one signal, at 102.76 p.p.m., in the anomeric region, and discrete signals, corresponding to ring carbon atoms, at 17.41 (C-6), 71.19 (C-5), 71.66 (a signal of a double integrated intensity for C-2, C-4), and 85.26 p.p.m. (C-3), and minor signals associated with the carbon atoms of the non-reducing unit at 71.50 (C-5'), 73.97 (C-4'), 75.90 (C-3'), and 103.45 p.p.m. (C-1).

Polycondensation of 4-*O*-acetyl-6-*O*-benzoyl-1,2-*O*-[1-(*exo*-cyano)ethylidene]-3-*O*-trityl-α-D-galactopyranose (**11**) in the presence of 0.1 mol of tritylium perchlorate under the standard conditions afforded, after the usual work-up[8] and

Zemplén deacetylation, a polysaccharide, $[\alpha]_D$ +99.2°, which contained, according to the ^{13}C-n.m.r. data, not less than 30% of α-linkages, distributed at random along the chain; the degree of polymerization amounted to merely 8, and the yield was 25%. On carrying out this polycondensation reaction under the optimum conditions described in the foregoing, at a pressure of 1.4 GPa, the polysaccharide, $[\alpha]_D$ −11.3° (*c* 1, water), was obtained in 55% yield. The ^{13}C-n.m.r. data showed it to be fully regular. In the anomeric region, the spectrum contained only one signal, at 105.1 p.p.m., which could only be assigned to the β-galactosidic linkages, and signals at 96.0–96.5 p.p.m., corresponding to α-galactose units, were altogether absent. The spectrum also contained some discrete signals at 62.12 (C-6), 69.59 (C-4), 71.51 (C-2), 75.87 (C-5), and 83.10 (C-3), which are full agreement with literature data[11], as well as minor signals at 69.85 (C-4′), 72.35 (C-2′), 73.85 (C-3′), and 76.23 (C-5′), corresponding to the carbon atoms of the galactopyranose unit at the non-reducing terminus. Methylation analysis showed that the polysaccharide contained solely (1→3)-glycosidic linkages, and the average degree of polymerization was 22. Only 1,5-di-*O*-acetyl-2,3,4,6- and 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylgalactitol were formed in this analysis.



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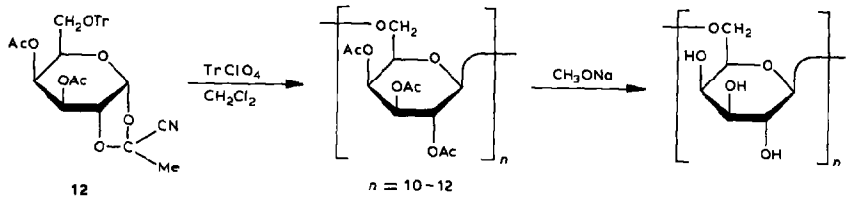
In order to elucidate the influence of high pressure on an increase in the yield and molecular weight of the polysaccharides produced, we conducted the synthesis of (1→6)-β-D-galactan. Polycondensation of 3,4-di-*O*-acetyl-1,2-*O*-[1-(*exo*-cyano) ethylidene]-6-*O*-trityl-α-D-galactopyranose (**12**) under the usual conditions[10] afforded a fully stereospecific polysaccharide, although its yield was but 40%, and the degree of polymerization was quite small (~8). In contrast, on carrying out the same reaction at a pressure of 1.0 GPa under the conditions already described, a stereospecific polysaccharide having a degree of polymerization of 10–12 was obtained in a yield of >90%.

The experiments on polycondensation of trityl ethers and cyanoethylidene



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derivatives of sugars, carried out at high pressure, showed that polycondensation under these conditions becomes highly stereospecific, and the yields and molecular weight of polymers increase. Thus, the scope of this thus-far unique, general method for the synthesis of polysaccharides is considerably extended, allowing it to be used, in particular, for the preparation of stereoregular glucans and galactans having glycosidic linkages through the secondary atoms of the monosaccharide units.

The data reported herein on the influence of pressure on the stereochemical course of the glycosylation reaction seems not to be restricted to the trityl–cyanoethylidene condensation. According to preliminary data, the Helferich glycosylation is affected by pressure in a similar way.

EXPERIMENTAL

Dichloromethane and acetonitrile were distilled from P_2O_5, and, directly before utilization, twice from CaH_2. Optical rotations were measured with a Perkin–Elmer polarimeter, model 141, and melting points were determined in a Koffler apparatus. Thin-layer chromatography was performed on plates with a fixed layer of SiO_2 gel (Merck), and preparative chromatography was carried out on SiO_2 L 100/160 (CSSR). Experiments under pressure were performed in an apparatus, the scheme of which is shown in ref. 12. The structure of all compounds obtained was confirmed by elemental analysis and ^{1}H-n.m.r.-spectral data. ^{1}H-N.m.r. spectra were recorded with a Bruker WM-250 instrument for solutions in $CDCl_3$, with Me_4Si as an internal standard. ^{13}C-N.m.r. spectra were recorded with Bruker WM-250 and Bruker AM-300 instruments for solutions in Me_2SO or D_2O, with CH_3OH as an internal standard (50.15 p.p.m. with respect to Me_4Si). Infrared spectra were recorded with a UR-20 instrument (GDR).

*Synthesis of disaccharides. Methyl 3,4,6-tri-*O*-acetyl-2-*O*-trityl-α-*D*-glucopyranoside* (**3**). — To a solution of methyl 4,6-*O*-(phenylborylene)-α-D-glucopyranoside[13] (5 g, 18 mmol) in CH_2Cl_2 (200 mL) were added $TrClO_4$ (6.2 g, 18 mmol) and γ-collidine (2.65 mL, 20 mmol). The mixture was kept for 10 min at room temperature, and evaporated to dryness, and the residue was dried *in vacuo*, and dissolved in CH_2Cl_2 (100 mL); then, 1,3-propanediol (1.5 mL) was added, and the solution was evaporated to dryness. Benzene was added to the residue, and the precipitate was separated. The benzene solution was evaporated to dryness, C_5H_5N (50 mL) and $(CH_3CO)_2O$ (20 mL) were added, and the solution was kept over-night at room temperature. To the mixture was added methanol (20 mL), the mixture was evaporated to dryness, and the residue subjected to chromatography in benzene–ether (gradient elution), to give 7.2 g of a chromatographically homogeneous compound (74%). Recrystallization from hexane with addition of ethyl acetate yielded crystalline **3** (6.5 g, 66%); m.p. 152–153°, $[\alpha]_D$ +60° (*c* 1, $CHCl_3$); n.m.r. data: δ 7.2–7.5 (15 H, aromatic), 5.64 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), 4.75 (dd, 1 H $J_{4,5}$ 10 Hz, H-4), 4.12 (dd, 1 H, $J_{5,6}$ 5.5, $J_{6,6'}$ 12 Hz, H-6), 3.98 (dd, 1 H, $J_{6',5}$ 2.5 Hz,

H-6'), 3.88 (ddd, 1 H, H-5), 3.8 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 3.56 (dd, 1 H, H-2), 3.21 (s, OCH_3), and 2.02, 1.98, and 1.84 (3 s, 9 H, 3 OAc).

Anal. Calc. for $C_{32}H_{34}O_9$: C, 68.31; H, 6.09. Found: C, 68.10; H, 6.02.

Reactions under pressure. General procedure. — A solution of the 1,2-cyanoethylidene (0.2 mmol) and *O*-trityl (0.2 mmol) derivatives in CH_2Cl_2 was placed in a 3.6 mL Teflon ampoule; then a separately prepared solution of $TrClO_4$ (0.015–0.02 mmol) in CH_2Cl_2 was added, and the remaining volume was made up with pure solvent. The ampoule was quickly sealed hermetically, and immersed, if desired, in a cooling mixture at −75°, or in liquid nitrogen. Next, the ampoule was placed in the reaction vessel, and high pressure was applied. (The pressure of 1.4 GPa is attained in 8 min.) After a certain period of time (see Table I), the pressure was released, the contents of the ampoule were diluted with $CHCl_3$ (25 mL), and then CH_3OH (0.1 mL) and C_5H_5N (0.1 mL) were added, and the mixture was washed with water (5 × 10 mL). The solution was evaporated to dryness, and a disaccharide-containing zone was separated by preparative chromatography on SiO_2 gel in a gradient of benzene → ethyl acetate. By comparison of integrated intensities of signals of anomeric carbon atoms in the ^{13}C-n.m.r. spectrum of the disaccharide mixture obtained, the relative content of the 1,2-*trans*-/1,2-*cis*-linked disaccharides was obtained.

The following individual disaccharides were isolated from the product of the synthesis under a pressure of 1.4 GPa.

O-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→3)-1,2,4,6-tetra-*O*-acetyl-β-D-glucopyranose; m.p. 160–161°, $[\alpha]_D$ −28.5° (*c* 2, $CHCl_3$); lit.[14] m.p. 160°, $[\alpha]_D$ −29.3°.

Methyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3-di-*O*-acetyl-β-D-xylopyranoside, a syrup, $[\alpha]_D$ −37.5° (*c* 2, $CHCl_3$), lit.[7] a colourless syrup, $[\alpha]_D$ −35.6° (*c* 1.8, $CHCl_3$).

O-(2,3,4-Tri-*O*-acetyl-6-deoxy-β-D-glucopyranosyl)-(1→3)-1,2,4,6-tetra-*O*-acetyl-β-D-glucopyranose; m.p. 180–180.5° (abs. ether), $[\alpha]_D$ −15.0° (*c* 2, $CHCl_3$).

Anal. Calc. for $C_{26}H_{36}O_{17}$: C, 50.32; H, 5.85. Found: C, 50.41; H, 5,84.

Synthesis of polysaccharides. 1,2-O-[1-(exo-Cyano)ethylidene]-6-deoxy-α-D-glucopyranose (**8**). — To a solution of compound **7** (ref. 6; 1.93 g, 6.5 mmol) in abs. C_5H_5N (7 mL) was added 0.1N methanolic sodium methoxide solution (2.4 mL), and, after 5 min, the base was neutralized with M aqueous CH_3CO_2H solution (0.25 mL). The solution was evaporated, and, by chromatography in a gradient of $CHCl_3$ → $CH_3CO_2C_2H_5$ was isolated **8** (1.22 g, 89%); m.p. 81–82° (ether), $[\alpha]_D$ +75.5° (*c* 2, $CHCl_3$); ^{1}H-n.m.r. data: δ 5.78 (d, 1 H, $J_{1,2}$ 5 Hz, H-1), 4.25 (t, 1 H, $J_{2,3}$ 5 Hz, H-2), 3.79 (dd, 1 H, $J_{3,4}$ 7 Hz, H-3), 3.72 (dq, 1 H, $J_{4,5}$ 9 Hz, H-5), 3.32 (dd, 1 H, H-4), 1.9 (s, 3 H, C-CH_3), and 1.36 (d, 3 H, $J_{5,6}$ 6 Hz, C-CH_3).

Anal. Calc. for $C_9H_{13}NO_5$: C, 50.19; H, 6.09; N, 6.51. Found: C, 50.01; H, 6.02, N, 6.30.

1,2-O-[1-(exo-Cyano)ethylidene]-6-deoxy-3-O-trityl-α-D-glucopyranose (**9**). - A solution of **8** (0.25 g, 1.15 mmol) and $TrClO_4$ (0.4 g, 1.15 mmol) in abs. CH_2Cl_2 (5 mL) containing γ-collidine (0.17 mL) was kept for 45 min at 20°. The mixture was

diluted with $CHCl_3$ (50 mL), washed with water (5 × 20 mL), and evaporated to dryness, to give 0.39 g (73%) of chromatographically homogeneous **9**; m.p. 128–129° (ether), $[\alpha]_D$ −54.5° (*c* 2, $CHCl_3$); ^{1}H-n.m.r. data: δ 7.2–7.55 (15 H, aromatic), 5.68 (d, 1 H, $J_{1,2}$ 5 Hz, H-1), 4.01 (dd, 1 H, $J_{3,4}$ 3 Hz, H-3), 3.62 (ddd, 1 H, $J_{2,3}$ 3 Hz, H-2), 3.44 (m, 2 H, H-4,5), 1.74 (s, 3 H, C–CH_3), and 1.3 (d, 3 H, $J_{5,6}$ 6 Hz, C–CH_3).

Anal. Calc. for $C_{28}H_{27}NO_5$: C, 73.15; H, 5.95; N, 3.06. Found: C, 72.93; H, 5.91; N, 2.98.

*4-O-Acetyl-1,2-O-[1-(exo-cyano)ethylidene]-6-deoxy-3-O-trityl-α-*D*-glucopyranose* (**10**). — To a solution of **9** (0.5 g, 1.1 mmol) in C_5H_5N (5 mL) was added Ac_2O (3 mL) and the mixture was kept overnight at 20°. CH_3OH (10 mL) was added, the mixture evaporated, and the residue evaporated several times with toluene until no pyridine odour was perceivable to give 0.52 g (94%) of **10**; m.p. 97–98° (benzene), $[\alpha]_D$ −47.5° (*c* 2.5, $CHCl_3$); ^{1}H-n.m.r. data: δ 7–7.52 (15 H, aromatic), 5.56 (d, 1 H, $J_{1,2}$ 5 Hz, H-1) 5.22 (dt, 1 H, $J_{3,4}$ 2 Hz, H-4), 3.97 (dd, 1 H, $J_{2,3}$ 2.5 Hz, H-3), 3.51 (dq, 1 H, $J_{4,5}$ 9 Hz, H-5), 3.08 (m, 1 H, H-2), 1.93 (s, 3 H, OAc), 1.73 (s, 3 H, C–CH_3), and 1.27 (d, 3 H, $J_{5,6}$ 6 Hz, C–CH_3).

Anal. Calc. for $C_{30}H_{29}NO_6$: C, 72.16; H, 5.81; N, 2.81. Found: C, 71.98; H, 5.77; N, 2.90.

*(1→3)-6-Deoxy-*D*-glucan. Polycondensation under atmospheric pressure.* — The polycondensation was carried out under atmospheric pressure by the method described[15], starting from monomer **10** (499 mg, 1 mmol) in the presence of $TrClO_4$ (34 mg, 0.1 mmol) in abs. CH_2Cl_2 (5 mL), for 100 h at 20°. After the usual work-up of the mixture, the acetylated polysaccharide (200 mg) was dissolved in a mixture of dry $CHCl_3$ (2 mL) and abs. CH_3OH (6 mL), M methanolic sodium methoxide solution (1.4 mL) was added, and it was stirred for 65 h at 20°. The mixture was evaporated (no absorption band for the carbonyl group was observed in the i.r. spectrum of the dry residue), and the residue was subjected to gel chromatography on a column (85 × 1.1 cm; V_{total} 85 mL, V_0 38 mL) of Sephadex G-25 in M aqueous CH_3CO_2H. A high-molecular-weight fraction of the polysaccharide was collected in 38 to 48 mL of eluate, to give 93 mg (63%) of the substance, $[\alpha]_D$ +99.7° (*c* 1.2, Me_2SO). Analysis of the partially methylated[16] alditol acetates revealed only 1,5-di-*O*-acetyl-6-deoxy-2,3,4-tri-*O*-methylglucitol and 1,3,5-tri-*O*-acetyl-6-deoxy-2,4-di-*O*-methylglucitol in the ratio of 1/14. The ^{13}C-n.m.r. spectrum of the free polysaccharide in Me_2SO contained the signals for the anomeric carbon atoms at 99–100 and 102–104 p.p.m., in the ratio of 59/41.

*(1→3)-6-Deoxy-β-*D*-glucan. Polycondensation under high pressure.* — Solutions of monomer **10** (215 mg, 0.43 mmol) and $TrClO_4$ (6 mg, 17.5 μmol) in abs. CH_2Cl_2 were placed in a 3.6-mL Teflon ampoule. The ampoule was hermetically sealed, immersed in liquid nitrogen, and then placed in the high pressure vessel cooled to 0°, to which a pressure of 1.4 GPa was applied in 8 min, and then the reactor temperature was elevated to 20°. After 20 h, the ampoule was unsealed, and, after the usual work-up, 90 mg of the acetylated polysaccharide, insoluble in

$CHCl_3$–CH_3OH, was obtained. For deacetylation, the product was dissolved in abs. Me_2SO (2 mL), M methanolic sodium methoxide solution (1 mL) was added, and the mixture was stirred for 20 h at 20°, and dialyzed against distilled water. Free, stereoregular polysaccharide (52.5 mg, 77%) was formed as a fluffy precipitate; $[\alpha]_D$ −9.1° (*c* 0.44, Me_2SO). G.l.c.–m.s. analysis of the partially methylated[16] alditol acetates showed the presence of only 1,5-di-*O*-acetyl-6-deoxy-2,3,4-tri-*O*-methylglucitol and 1,3,5-tri-*O*-acetyl-6-deoxy-2,4-di-*O*-methylglucitol in the ratio of 1:25. The ^{13}C-n.m.r. spectrum of the product in Me_2SO contained only one signal in the anomeric region, at 102.76 p.p.m., which is typical for β-(1→3)-linked quinovose units.

(1→3)-D-Galactan. Polycondensation under atmospheric pressure. — The polycondensation of monomer **11** was carried out, starting from 330 mg (0.53 mmol) in the presence of $TrClO_4$ (21 mg, 0.06 mmol) in abs. CH_2Cl_2 (4 mL), for 110 h at 20°. After the usual work-up, the acetylated polysaccharide (160 mg) was deacylated (2 mL of abs. CH_3OH, 2 mL of M CH_3ONa), affording 22 mg (25%) of a polysaccharide with the degree of polymerization 8; $[\alpha]_D$ +99.2° (*c* 1.8, H_2O). The ^{13}C-n.m.r. spectrum of the free polysaccharide in D_2O contained, in the anomeric region, signals at 105.29 and 96.47 p.p.m, typical for β-(1→3)- and α-(1→3)-linked galactose units.

(1→6)-β-D-Galactan. Polycondensation under atmospheric pressure. — The polycondensation[10] of monomer **12** was carried out starting from 560 mg (1 mmol) in the presence of $TrClO_4$ (34 mg, 0.1 mmol) in abs. CH_2Cl_2 (10 mL) for 100 h at 20°. After the usual work-up, the polycondensation product (300 mg) was deacylated (14 mL of abs. CH_3OH, 4 mL of M CH_3ONa) for 72 h at 20°. Chromatography on Sephadex G-25 afforded two fractions, 23 mg (13%) and 49 mg (30%); $[\alpha]_D$ +8.2° (*c* 1.2, H_2O) and $[\alpha]_D$ +30.8° (*c* 2.6, H_2O). The ^{13}C-n.m.r. spectrum of the first fraction of the free galactan in D_2O contained, in the anomeric region, one signal at 104.5 p.p.m. The degree of polymerization (equal to 8) was determined on the basis of the results of methylation analysis.

(1→3)-β-D-Galactan. Polycondensation under high pressure. — The polycondensation of monomer **11** was carried out under the conditions already described for monomer **10**, starting from 186 mg (0.3 mmol) of monomer **11** in the presence of $TrClO_4$ (4.1 mg, 12 μmol) in abs. CH_2Cl_2 (3.6 mL). The reaction was terminated by addition of CH_3OH and C_5H_5N, and the acylated polysaccharide was precipitated three times from chloroform with hexane. The precipitated dry residue (100 mg, 96%) was dissolved in a mixture of dry $CHCl_3$ (2 mL), abs. CH_3OH (2 mL), and M CH_3ONa–CH_3OH (2 mL), and stirred for 40 h at 20°. Gel chromatography on Sephadex G-25 afforded a fraction of free (1→3)-β-D-galactan (27 mg, 55%); $[\alpha]_D$ −11.3° (*c* 1, H_2O), with a degree of polymerization, determinecd by methylation analysis, equal to 22. The ^{13}C-n.m.r. spectrum contained, in the anomeric region, only one signal, at 105.1 p.p.m.

(1→6)-β-D-Galactan. Polycondensation under high pressure. — The polycondensation of monomer **12** was carried out as described in the synthesis of (1→3)-β-

D-galactan, without preliminary freezing of the ampoule in liquid nitrogen and cooling of the reaction vessel to 0°. Starting from 140 mg (0.25 mmol) of **12**, after the usual work-up, deacetylation, and gel chromatography on Sephadex G-25, the polysaccharide was separated into two fractions: fraction 1, 23.0 mg (56%), $[\alpha]_D$ +34.5° (*c* 1, H_2O); fraction 2, 16.5 mg (40%), $[\alpha]_D$ +30.0° (*c* 1.4, H_2O). The ^{13}C-n.m.r. spectra of the two fractions were identical to one another, and contained signals at 69.90, 70.55, 73.89, 74.97, 104.58 (C-4, C-6, C-2, C-3, C-5, and C-1). Methylation analysis showed that the average degree of polymerization for the first fraction was 12, and that for the second fraction was 10.

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