SOME KINETIC CHARACTERISTICS OF GLYCOSYLATION AT HIGH PRESSURES

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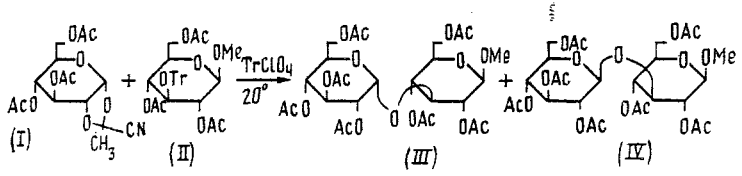
The effect was studied of pressure up to 1400 MPa on the stereospecificity of glycosylation during the condensation of 1,2-O-exocyanoethylidene-3,4,6-tri-O-acetyl-α-D-glucopyranose (I) with 2,4,6-tri-O-acetyl-3-O-trityl-β-methyl-D-glucopyranoside (II) in the presence of tritylium perchlorate, as a result of which a mixture of disaccharides containing an α- and β-glycoside bond is formed. It was shown that in the liquid phase (up to 1300 MPa) there is a smooth increase in the stereospecificity of glycosylation ($\Delta\overline{V}^{\neq}_{\beta,\alpha}$ = −5 cm³/mole) and in the rate of glycosylation ($\Delta V^{\neq}_{0}$ = −8 cm³/mole) as the pressure increases. Upon reaching a pressure that causes crystallization of the solvent (CH_2Cl_2, 1400 MPa, 20°C) the stereospecificity and glycosylation rate increase sharply and under these conditions the reaction takes place almost completely stereospecifically.

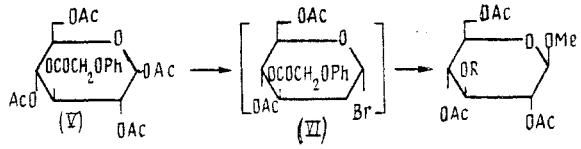
As was established earlier [1], the stereospecificity of the formation of the glycoside bond during trityl-cyanoethylidene condensation [2] increases with an increase in pressure. Recently it was noted that the ratio of the β-isomer to the α-isomer (β/α) undergoes a drastic increase after the solvent (CH_2Cl_2) is transformed to the solid phase. However, the glycosylation reaction where this effect was seen [3] led to a high β-isomer content (96.5%) even before solidification of the solvent; therefore, it was difficult to observe the effect of the solvent phase transition. In this article we present and discuss more detailed results of studies where such an effect was observed in a very clear form and was briefly reported in [4].

The effect of pressure on the stereospecificity of glycosylation was studied during the condensation of 1,2-O-exocyanoethylidene-3,4,6-tri-O-acetyl-α-D-glucopyranose (I) with 2,4,6-tri-O-acetyl-3-O-trityl-β-methyl-D-glucopyranoside (II) in the presence of tritylium perchlorate, which led to formation of a mixture of disaccharides containing an α- (III) and β-glycoside bond (IV)



(1)

Synthesis of the O-trityl derivative (II) was carried out starting with 1,2,4,6-tetra-O-acetyl-3-O-phenoxyacetyl-D-glucopyranose (V), described by us in [5], which via the formation of the bromide (VI) (not isolated as such) was transformed into the 3-O-phenoxyacetyl derivative (VII). Selective dephenoxyacetylation led to derivative (VIII), by tritylation of which the glycoside (II) was obtained



R = $OCOCH_2OPh$ (VII), H (VIII).

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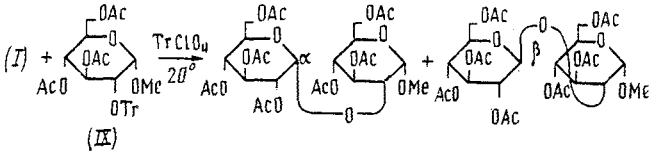
TABLE 1. Formation of β- and α-Linked (1 → 3) Disaccharides at 20°C and Various Temperatures (0.2 mmole (I) and (II) and 0.0145 mmole $TrClO_4$)

Expt.	p, MPa	Time, h	CH_2Cl_2, ml	Yield, % (α+β)	β, % (α+β)	α, % (α+β)	β/α	$k \cdot 10^3$ (mole fraction)$^{-1}$. sec^{-1}
1	0.1	100	3.8	60.0	48	52	0.92	1.25
2	1400*	20	3.8	69.3	96	4	24	9.4
3	1400*	4	3.8	49.6	95	5	19	20.6
4	0.1	4	2.3	11.2	48	52	0.92	1.6
5	200	4	2.3	15.8	56.6	43.4	1.3	2.4
6	400	4	2.3	–	61	39	1.6	–
7	600	4	2.3	22.0	68	32	2.1	3.6
8	800	4	2.3	34.5	73	27	2.7	5.8
9	1000	4	2.3	40.8	75	25	3.0	8.7
10	1200	4	2.3	47.25	77	23	3.3	11.4
11	1300	4	2.3	52.3	78	22	3.5	13.9
12	1400*	4	2.3	68.5	95	5	19.0	27.6
13	1400**	4	3.8		100	0		–

*Freezing of the reaction mixture was observed along with a sharp drop in pressure.
**Reactor cooled to 5°C, pressure of 1400 MPa applied, freezing of the reaction mixture registered, after which the reactor temperature reached 20°C.

The results obtained from reaction of (I) and (II) are given in Table 1. Judging from the above data the ratio β/α does not depend on the length of the reaction (yield of isomers). At atmospheric pressure (0.1 MPa) the β/α ratio remains constant as the reaction time is increased from 4 to 100 h and, at the same time, the isomer yield increases from 11 to 60% (expts. 1 and 4). Even at p = 1400 MPa when the solvent transforms to the solid phase increasing the reaction time from 4 to 20 h hardly affects the isomer ratio (expts. 2 and 3). This makes it possible to explain the effect of pressure on the β/α ratio by comparing experiments that have a constant reaction time but varying isomer yields that grow with an increase in pressure (expts. 4-12). From the data in the table it also follows that the reaction, which is entirely nonstereospecific at atmospheric pressure when the α-isomer is formed in the larger amount (β/α = 48/52), changes its character as the pressure increases, as a result of which the β-isomer content is significantly increased. At 1300 MPa the β/α ratio is 78/22 having increased 4 times as compared with atmospheric pressure. However, it is significant that with an increase in pressure to 1400 MPa, i.e., by 100 MPa, an especially sharp jump follows and the β/α ratio increases to 95/5 for a five-fold increase. It is precisely in the range 1350-1400 MPa that the solvent freezes (which is clearly registered by a sharp pressure drop of 52 MPa). It follows that the sharp increase in stereospecificity of the reaction is connected with the phase transition.

Figure 1 shows a plot of log β/α vs. pressure of two reactions, reaction (1) and the similar reaction of (I) with 3,4,6-tri-O-acetyl-2-O—trityl-α-methyl-D-glucopyranoside (IX)(2), which was studied in [3]



(2)

As can be seen, reaction (1) (Fig. 1, curve 2) at first is much less stereospecific ($\Delta V^{\neq}_{\beta,\alpha}$ = −5 cm³/mole) than reaction (2) (Fig. 1, curve 1,, $\Delta \bar{V}^{\neq}_{\beta,\alpha}$ −8.5 cm³/mole) which spasmodically changes its nature during the phase transition finally reaching a high degree of stereospecificity.

In Fig. 2 the data of Table 1 are presented as plots of yield of α- (curve 1) and β-isomer (curve 2) vs. pressure. As can be seen, in the liquid phase as the pressure increases the yield of both isomers increases but the β-isomer yield increases significantly more. At the solvent phase transition pressure (1400 MPa) the curve sharply changes its character, the content of β-isomer increasing greatly and that of the α-isomer sharply decreasing.

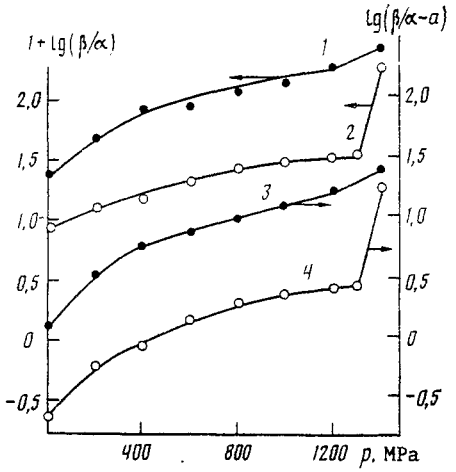
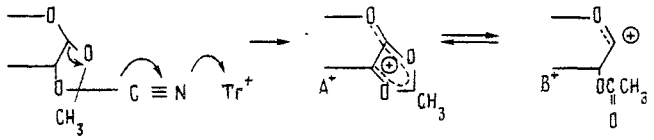


Fig. 1. Logarithm of ratio of stereospecific isomers in the glycosylation reaction vs. pressure: 1) reaction (2) [3], 2) reaction (1). Plot of $\log[(\beta/\alpha) - a]$ vs. pressure: 3) reaction (2), [3], a = 1; 4) reaction (1) a = 0.7.

From a plot of log k vs. pressure (Fig. 3) (k is the glycosylation rate constant calculated from the second order equation) the volume effect is evaluated for activation of reaction (1) which is characterized by an increase in the overall glycosylation rate with an increase in pressure. It was -8 cm^3/mole in the range below 1300 MPa. Increasing the pressure from atmospheric to 1300 MPa accelerates the reaction 8.7 times and in the region of solvent crystallization a 100 MPa pressure increases the rate 2 times. The analogous relation for reaction (2) is shown in Fig. 3 for comparing the effect of pressure on the overall glycosylation rate.

We will examine some kinetic features of reactions (1) and (2) from the point of view of the glycosylation mechanism proposed earlier [1, 3]. This mechanism postulates the rapid establishment of equilibrium between the bicyclic 1,2-acyloxonium cation A^+, formed by attack of the trityl ion (from $TrClO_4$) of the cyanoethylidene derivative (I), and the monocyclic glycosyl cation B^+, obtained during the isomerization of A^+.



Reaction of A^+ with (II) leads to the formation of only the β-isomer (k_β) and the reaction of (II) with B^+ gives both β-(k_β') and the α-isomer (k_α). Then the following expression is obtained for the β/α ratio

$$\frac{\beta}{\alpha} = \frac{k_\beta}{k_\alpha} K_{BA} + \frac{k_\beta'}{k_\alpha} \qquad (3)$$

where $K_{BA} = [A^+]/[B^+]$ is the equilibrium constant between A^+ and B^+. Further, it is assumed that the rate constants k_β, k_α, and k_β' change equally with an elevation of pressure while the ratios k_β/k_α and k_β'/k_α remain constant. In the case of reaction (2) it is also assumed that the probability of attack by the O-trityl derivative of the monocyclic cation B^+ on both sides of the ring is the same, i.e., $k_\beta' = k_\alpha$, and from equation (3) we obtain

$$\lg K_{BA} = \lg\left(\frac{\beta}{\alpha} - 1\right) - \lg\frac{k_\beta}{k_\alpha} \qquad (4)$$

In this case, taking into account the constancy of k_β/k_α a plot of $\log[(\beta/\alpha) - 1]$ vs. p reflects the change in log K_{BA} with an increase in p, which is determined by the magnitude of

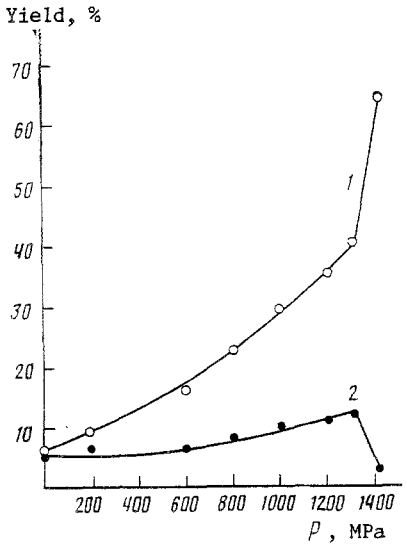


Fig. 2

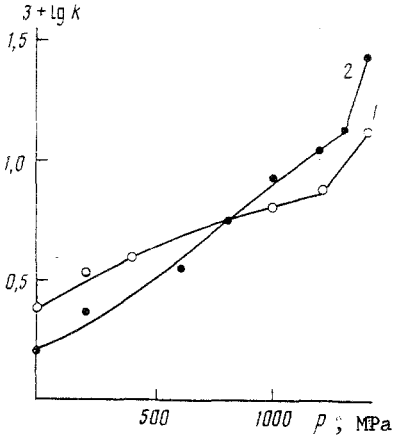


Fig. 3

Fig. 2. Yields of β-isomer (1) and α-isomer (2) vs. pressure: 20°C, 4 h.

Fig. 3. Logarithm of specific glycosylation rate vs. pressure: 1) for reaction (2) [3], 2) reaction (1).

the volume change during the conversion reaction $B^+ \rightarrow A^+$. It appears that this value exists in congruity with the expected decrease of the volume during the formation of a new ring ($\Delta V_{BA} = -11$ cm^3/mole) [3].

In addition, for reaction (1) the ratio β/α is even somewhat smaller than 1 (β/α = 48/52) at atmospheric pressure. Also, as was noted, the value of β/α in this case increases more slowly as the pressure increases in the liquid phase. These differences cannot be related to the value of K_{BA} since the reactions are carried out under the same conditions including the same cyanoethylidene derivative (I). Consequently, one and the same cations A^+ and B^+ participate in the equilibrium conversion. Therefore, for reaction (1) $(k_\beta'/k_\alpha) < 1$, i.e., during attack by the O-trityl derivative of cation B^+ formation of the α-isomer is more preferred. It appears that if the ratio k_β'/k_α is assumed to be equal to 0.7 and not 1 for reaction (1), i.e., it is decreased in all by 30%, $\log[(\beta/\alpha) - 0.7]$ vs. pressure is identical to the function $\log[(\beta/\alpha) - 1]$ for reaction (2).

Further, by using experimental values of β/α for both reactions at atmospheric pressure and assuming values of k_β'/k_α the relation $(k_\beta/k_\alpha)K_{BA}$ can be calculated from Eq. (3). For reaction (1) the latter is 0.22 and for (2) it is 1.33. Consequently, the value of k_β/k_α is 6 times larger for reaction (2) than for reaction (1).

Thus, a change in the structure and reactivity of the aglycone only slightly affects the relative rate of formation of the α- and β-isomer during attack on the monocyclic cation B^+ and has a very strong effect on the relative formation rate of the same isomers in that case where the β-isomer is obtained from the bicyclic cation A^+. From the point of view of the examined mechanism the decrease of k_β/k_α during the transition from reaction (2) to reaction (1) causes total loss of stereospecificity at atmospheric pressure.

The reactivity of the aglycone (II) in glycosylation is less than that of the aglycone participating in reaction (2). This can be visualized by comparing the specific reaction rates of (1) and (2) at atmospheric pressure. Thus, for reaction (1) $k \cdot 10^3 = 1.6$ (Table 1, expt. 4) and for reaction (2) $k \cdot 10^3 = 2.5$ [3] although the concentration of catalyst, which is not counted in the calculation of k, is 1.5 times greater in reaction (1) than in (2). Evidently, the decrease in the reactivity of the aglycone (II) is caused by the markedly larger difference in its reaction rates with cations A^+ and B^+.

In conclusion we will examine experiment 13 (Table 1). If the ampul, cooled to −196°C, is placed in the reactor at 5°C (to avoid a reaction taking place at the time of the pressure drop), the pressure raised to 1400 MPa, the reactor brought to room temperature (2 h), and the reaction mixture held for 20 h, then the disaccharide obtained consists only of the β-isomer.

The most important result of the kinetic study of the trityl-cyanoethylidene condensation presented in this article is the sharp increase in stereospecificity during the phase transition of the solvent. This result is of great interest coming within the framework of the studied reaction. In the present case it is possible that this is connected with an increase in the stability of the bicyclic cation A^+ and the sharp inhibition of its conversion to the monocyclic cation B^+ in the frozen solution. On the other hand, since all the starting compounds (with the exception of the solvent) formation of a multiphase solid system and decrease of the overall reaction rate would be expected. Meanwhile the overall reaction rate (Table 1, Fig. 3) increases so that all the phenomena on the whole are not trivial and deserve the most intense attention.

EXPERIMENTAL

CH_2Cl_2 was distilled over P_2O_5 and twice over CaH_2. Chemical shifts in the NMR spectra of ^1H and ^{13}C were determined on Bruker WM-250 and Bruker AM-300 instruments in $CDCl_3$ relative to tetramethylsilane. Specific rotation was determined on a Perkin–Elmer 141 polarimeter and the melting points in a Koffler block. TLC was carried out on glass plates with a fixed layer of SiO_2 (Merck) and preparative column chromatography in SiO_2 grade 100/160 (Czechoslovak SSR). Experiments under pressure were carried out in an apparatus described in [6]. Pressure was measured from the change in resistance of a manganin coil placed in the reactor inlet. Phase transitions of CH_2Cl_2 and of the reaction mixture were registered from a determined sharp pressure drop as described in [7]. The experiments were timed from the moment of reaching the necessary pressure.

2,4,6-Tri-O-acetyl-3-O-phenoxyacetyl-β-methyl-D-glucopyranoside (VII). Six mmoles (2.89 g) of 1,2,4,6-tetra-O-acetyl-3-O-phenoxyacetyl-D-glucopyranose (V) [5] is dissolved in a mixture of 25 ml absolute C_6H_6 and 50 ml of a saturated solution of HBr in AcOH. After 2 h the mixture is diluted with 100 ml $CHCl_3$, washed with water (200 ml), then with a saturated solution of $NaHCO_3$ (2 × 200 ml), again with water, and evaporated. The chromatographically homogeneous halogenose (VI) obtained in the residue is dissolved in a mixture of 40 ml absolute CH_3OH in 10 ml ofabsolute CH_2Cl_2, 2.7 g freshly prepared Ag_2O added, the solution stirred with a magnetic stirrer for 18 h (20°C), after which the precipitate is separated, the filtrate boiled to dryness, the residue chromatographed on SiO_2 (elution gradient, C_6H_6–Et_2O). We obtained 2.25 g of chromatographically pure homogeneous glycoside (VII), mp 96-97°C (alcohol–ether–petroleum ether), $[\alpha]_D = -16°$ (c 2, $CHCl_3$). NMR spectrum ($CDCl_3$, δ, ppm): 6.8-7.4 (5H, aromatic protons), 5.31 d.d. (1H, H^3, $J_{3,4}$ = 9.5 Hz), 5.14 d.d (1H, H^4, $J_{4,5}$ = 9.5 Hz), 5.03 d.d. (1H, H^2, $J_{2,3}$ = 9.5 Hz), 4.57 s (CH_2), 4.46 d (1H, H^1, $J_{1,2}$ = 8 Hz), 4.31 d.d (1H, H^6, $J_{6,6'}$ = 12.5 Hz), 4.16 d.d (1H, $H^{6'}$, $J_{6,5}$ = 2.5 Hz), 3.73 m (1H, H^5, $J_{5,6}$ = 4.5 Hz), 3.52 s (–OCH), 2.13 s, 2.02 s, 1.98 s (9H, 3OAc). Found: C 55.38, H 5.71%, $C_{21}H_{26}O_{11}$. Calculated: C 55.5, H 5.77%.

2,4,6-Tri-O-acetyl-β-methyl-D-glucopyranoside (VIII). A solution of 2.17 g (4.8 mmoles) of (VII) in 2.8 ml absolute CH_2Cl_2 is cooled to −20°C, diluted with 18 ml of CH_3OH, saturated with NH_3 at 0°C, and then cooled to −20°C. The mixture is held for 40 min at −20°C, cooled to −40°C, and neutralized to pH 6.5 with a 20% aqueous solution of HCl. The solution obtained is evaporated almost to dryness, 50 ml $CHCl_3$ and 10 ml water added to the residue, and the solution transferred to a separatory funnel. The organic layer is washed with 10 ml water, evaporated, and chromatographed on SiO_2 (elution gradient, C_6H_6–Et_2O). Obtained 0.84 g (55%) of (VIII), mp 102-103°C (C_6H_6), $[\alpha]_D = -43.9°$ (c, 2.8, $CHCl_3$). NMR spectrum ($CDCl_3$, δ, ppm): 4.96 d.d (1H, H^4, $J_{4,5} = J_{3,4}$ = 9.5 Hz), 4.86 d.d (1H, H^2, $J_{2,3}$ = 9.5 Hz), 4.37 d (1H, H^1, $J_{1,2}$ = 8 Hz), 4.28 d.d. (1H, H^6, $J_{6,6'}$ = 12 Hz), 4.15 d.d (1H, $H^{6'}$, $J_{5,6'}$ = 2.5 Hz), 3.72 m (H^3, 1H), 3.64 m (1H, H^5, $J_{5,6}$ = 5 Hz), 3.50 s ($-OCH_3$), 3.25 sh. s (1H, –OH), 2.14 s, 2.12 s, 2.08 s (9H, 3OAc). Found: C 48.88; H 6.22%. $C_{13}H_{20}C_9$. Calculated: C 48.75; H 6.29%.

2,4,6-Tri-O-acetyl-3-O-trityl-β-methyl-D-glucopyranoside (II). The hydroxyl derivative of (VIII) 0.8 g (2.5 mmoles) and 1.0 g (2.9 mmoles) of $TrClO_4$ (dried beforehand in vacuo for 30 min) are dissolved in 20 ml absolute CH_2Cl_2 containing 0.44 ml γ-collidine and held at 20°C for 2 h, after which the solution is diluted with 4 ml 50% aqueous pyridine and 100 ml $CHCl_3$. The mixture is washed with water (5 × 40 ml) and evaporated. After chromatography on SiO_2 (elution gradient, C_6H_6–Et_2O) 1.34 g (95.3%) of chromatographically homogeneous tritylated glycoside of (II) was obtained, mp 175-176°C (Et_2O–C_5H_{12}), $[\alpha]_D = -11.7°$ (c 2, $CHCl_3$). NMR spectrum ($CDCl_3$, δ, ppm): 7.2-7.45 (15H, aromatic protons), 5.18 d.d (1H, H^4, $J_{4,5} = J_{3,4}$ = 9

Hz), 5.12 d.d (1H, H^2, $J_{2,3}$ = 9 Hz), 4.16 m (2H, H^6, H^6), 3.93 d (1H, H^1, $J_{1,2}$ = 8 Hz), 3.41 d.d (1H, H^3, $J_{3,4}$ = 9 Hz), 3.39 s ($-OCH_3$), 3.21 m (1H, H^5, $J_{5,6}$ = 4 Hz, $J_{5,6'}$ = 2 Hz), 2.07 s, 1.52 s, 1.47 s (9H, 3OAc). Found: C 68.02; H 6.04%. $C_{32}H_{34}O_9$. Calculated: C 68.32; H 6.09%.

Reactions under Pressure. General Procedure. A solution of the following mixture is placed in a Teflon ampul: 0.2 mmole (72 mg) of 1,2-O-exocyanoethylidene-3,4,6-tri-O-acetyl-α-D-glucopyranose (I) [8], 0.2 mmole (113 mg) of the O-trityl derivative of (II) in CH_2Cl_2, and a separately prepared solution of $TrClO_4$ 0.0145 mmole (5 g) in the same solvent. The solution was brought to volume with pure solvent. The ampul is hermetically sealed and placed in liquid nitrogen (to prevent the reaction taking place at atmospheric pressure). Then the ampul is placed in the reactor where high pressure is applied (1400 MPa reached in 8 min). After a given time (cf. Table 1) the contents are diluted with 25 ml $CHCl_3$, 0.1 ml MeOH and 0.1 ml C_5H_5N added, and the solution washed with water (5 × 10 ml). The solution is evaporated to dryness and dried in vacuo. The mixture obtained is acetylated with 2 ml of $(CH_3CO)_2O$ in 2 ml C_5H_5N for 20 h at room temperature, MeOH is added to the mixture and it is evaporated in vacuo to dryness. Preparative chromatography in SiO_2 in the system benzene–ethylacetate (elution gradient) separated a zone containing disaccharides. The relative content of the 1,2-trans- and 1,2-cis disaccharides (β- and α-isomers) was determined with ^{13}C NMR spectroscopy of the disaccharide fraction by comparing integrated intensities of the signals from the anomeric carbon atoms of the unreduced chain (101.0 and 96.13 ppm, respectively).

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