Partial methanolysis and hydrazinolysis of some derivatives of 1,6-anhydro-β-D-glucopyranose

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The effect of polar and steric factors of the substituent attached to carbonyl group of the ester on the course of hydrolysis of per-*O*-butyryl and per-*O*-palmitoyl derivatives of 1,6-anhydro-β-D-glucopyranose has been followed. The stability and electron structure of 1,6-anhydro-β-D-glucopyranose as well as of neutral and protonated forms of its acyl derivatives were calculated by the PCILO quantum-chemical method. The experimental data and the results of calculations indicate that different reactivity of acyloxy group at C-3 in dependence on the reaction conditions (in methanolysis the lowest, in hydrazinolysis the highest) is due to the possible stabilization of the activated complex by the O-3···H···O-1 hydrogen bond. The preparation of all 12 mono- and di-*O*-butyryl and -palmitoyl derivatives of 1,6-anhydro-β-D-glucopyranose is described.

Исследовался эффект полярных и стерических факторов заместителя, присоединенного к карбонильной группе сложного эфира в ходе гидролиза пер-*O*-бутирил- и пер-*O*-пальмитоил-производных 1,6-ангидро-β-D-глюкопиранозы. Устойчивость и электронное строение 1,6-ангидро-β-D-глюкопиранозы, а также нейтральной и протонированной форм ее ацил-производных были рассчитаны с помощью квантовохимического метода PCILO. Экспериментальные данные и результаты расчетов говорят о том, что различная реакционноспособность ацилокси-группы на C-3 в зависимости от реакционных условий (наименьшая при метанолизе и наибольшая при гидразинолизе) является следствием вероятной стабилизации активированного комплекса посредством водородной связи O-3···H···O-1. Описано получение всех 12 моно- и ди-*O*-бутирил- и -пальмитоил-производных 1,6-ангидро-β-D-глюкопиранозы.

In our previous paper [1] we dealt with partial methanolysis and hydrazinolysis of acetyl and benzoyl derivatives of 1,6-anhydro-β-D-glucopyranose (*I*). We found that the stability of the individual ester groups was affected mainly by steric arrangement of the molecule *I*, reaction conditions, and the acyl derivative (higher stability of benzoyl than acetyl derivative).

To investigate in more detail the effect of the aforementioned factors on the course of methanolysis and hydrazinolysis, we applied PCILO quantum-chemical calculations. We followed the possibilities of stabilization of the protonated monohydroxy ester of ortho acid, forming in the course of methanolysis, as well as the effect of the size of the alkyl attached to carbonyl group on the charge distribution around the reaction centre. These aspects were studied with 1,6-anhydro-β-D-glucopyranose, its per-*O*-acetyl derivative, and 3-*O*-substituted (acetyl, propionyl, butyryl, valeryl) derivatives of 2,4-di-*O*-acetyl-1,6-anhydro-β-D-glucopyranose, considering both neutral and protonated forms. For experimental purposes butyryl and palmitoyl derivatives of 1,6-anhydro-β-D-glucopyranose, *i.e.* derivatives with increasing length of the alkyl group of the acyl were chosen as model compounds. The effect of these substituents on the course of methanolysis, hydrazinolysis, and esterification was followed. Acylations were carried out with butyric and palmitic anhydrides. The course of methanolysis was monitored by means of GLC and TLC and the results obtained are summarized in Tables 1 and 2. The results of methanolysis and hydrazinolysis

Table 1

Percentage of the particular acyl derivatives at the hydrolysis of 1,6-anhydro-2,3,4-tri-*O*-butyryl-β-D-glucopyranose in methanolic hydrogen chloride

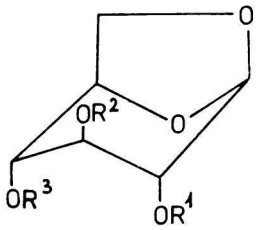
Time	Position of the butyryl group							
h	0	4	2	3	2,3	2,4	3,4	2,3,4
2	0	0	0	1.4	10.0	2.1	7.1	79.4
4	0	0.4	0.3	1.4	11.0	2.2	8.0	76.6
6	Traces	0.5	0.4	3.6	13.7	2.4	10.0	69.5
8	Traces	0.6	0.5	4.6	16.6	2.7	12.0	62.8
24	2.8	3.0	2.51	25.5	25.4	3.4	17.1	20.8
48	7.5	4.1	2.7	52.3	19.1	2.6	10.1	1.6
72	21.2	3.7	2.0	57.3	11.7	Traces	4.8	0.4

Table 2

Percentage of the particular acyl derivatives at the hydrolysis of 1,6-anhydro-2,3,4-tri-*O*-butyryl-β-D-glucopyranose in hydrazine hydrate

Time	Position of the butyryl group							
h	0	4	2	3	2,3	2,4	3,4	2,3,4
1	0	2.0	1.0	1.0	7.9	7.8	6.8	73.4
2	0	5.6	3.1	3.4	11.9	11.8	11.0	53.1
4	1.8	13.9	7.8	9.3	14.1	18.1	14.9	20.1
6	2.7	18.0	10.3	14.1	11.7	20.1	11.7	11.4
24	14.3	29.4	14.4	24.6	3.3	12.4	3.7	Traces
48	17.1	28.4	14.2	27.2	1.8	9.9	1.5	0

of *IX* were evaluated on the basis of preparative chromatographic separation of the reaction mixtures. Choice of suitable reaction conditions and separation methods made possible to separate all mono- and di-*O*-butyryl and -palmitoyl derivatives (Scheme 1). 3-*O*-Butyryl (*VI*) and 3-*O*-palmitoyl (*XIV*) derivatives



I	$R^1 = R^2 = R^3 = OH$	*IX*	$R^1 = R^2 = R^3 = Pal$
II	$R^1 = R^2 = R^3 = Bu$	*X*	$R^1 = H;\ R^2 = R^3 = Pal$
III	$R^3 = H;\ R^1 = R^2 = Bu$	*XI*	$R^3 = H;\ R^1 = R^2 = Pal$
IV	$R^2 = H;\ R^1 = R^3 = Bu$	*XII*	$R^2 = H;\ R^1 = R^3 = Pal$
V	$R^1 = H;\ R^2 = R^3 = Bu$	*XIII*	$R^1 = R^2 = H;\ R^3 = Pal$
VI	$R^1 = R^3 = H;\ R^2 = Bu$	*XIV*	$R^1 = R^3 = H;\ R^2 = Pal$
VII	$R^1 = R^2 = H;\ R^3 = Bu$	*XV*	$R^2 = R^3 = H;\ R^1 = Pal$
VIII	$R^2 = R^3 = H;\ R^1 = Bu$		

(Bu = butyryl; Pal = palmitoyl)

Scheme 1

were prepared also by the procedure similar as used in the preparation of 3-*O*-acetyl-1,6-anhydro-β-D-glucopyranose [2]. The positions of acyl groups were proved by ^{1}H NMR spectroscopy (Table 3). The results obtained show that the reaction conditions (methanolic hydrogen chloride and hydrazine hydrate) had determining influence on stability of the ester linkage. The inductive effect of the alkyl group attached to carbonyl carbon of the ester is complementary.

The different stability of the ester bond at C-3 to methanolysis and hydrazinolysis can be explained by different mechanisms of these reactions. In methanolysis addition of a proton to the carbonyl oxygen takes place first, then methanol is added under formation of a protonated monohydroxy ester of ortho acid. In the next step the proton is transferred to the ester oxygen, the result of which is the tautomeric form of the protonated monohydroxy ester of ortho acid. Stabilization of this activated complex by the O-3···H···O-1 hydrogen bond makes its decomposition and thus also the methanolysis of the acyloxy group on C-3 slower. This assumption was confirmed also by the PCILO calculations of 2,3,4-tri-*O*-acetyl-β-D-glucopyranose protonated in 2,3, and 4 positions, pointing to the highest stability of the position C-3. The energy

Table 3

Chemical shifts in ^{1}H NMR spectra of butyryl and palmitoyl derivatives of 1,6-anhydro-β-D-glucopyranose

Compound	H-1	H-2	H-3	H-4	H-5	H-6 *endo*	H-6 *exo*	OH
II	5.38	4.67	4.81	4.50	4.63	4.15	3.73	*
III	5.33	4.63	4.79	3.40	4.58	4.10	3.72	*
IV	5.34	4.63	3.70	4.53	4.58	4.18	3.68	4.80
V	5.34	3.60	4.79	4.48	4.57	4.04	3.70	*
VI	5.31	3.39	4.75	3.58	4.53	4.06	3.69	4.30 4.14
VII	5.27	3.39	3.64	4.55	4.47	4.07	3.64	3.07
VIII	5.28	4.49	3.66	3.57	4.52	4.10	3.64	3.37
IX	5.45	4.59	4.84	4.62	4.60	4.10	3.80	*
X	5.46	3.50	4.79	4.68	4.59	4.12	3.82	3.23
XI	5.43	4.61	4.80	3.56	4.59	4.10	3.82	*
XII	5.47	4.57	3.73	4.67	4.59	4.19	3.76	*
XIII	5.50	3.77	3.56	4.73	4.58	4.24	3.81	*
XIV	5.45	3.55	4.88	3.62	4.59	4.06	3.81	3.42
XV	5.46	4.60	3.88	4.21	4.59	4.24	3.88	3.65

* Not observed.

Chemical shifts for butyryl and palmitoyl groups in the individual compounds varied in the range of $\delta = \pm 0.02$ ppm:

palmitoyl group: ^{1}H NMR: 0.88 t (CH_3), 1.28 s (CH_2/n), 1.62 t (CH_3—$\underline{CH_2}$), 2.35 t (—$\underline{CH_2}$—CO);

butyryl group: ^{1}H NMR: 0.95 t (CH_3), 1.63 q (CH_3—CH_2—), 2.35 t ($\underline{CH_2}$—CO).

of the protonated form of C-3 is lower owing to stabilization by the O-3···H··· ···O-1 hydrogen bond which may exist due to the suitable distance (294 pm) between the oxygen atoms O-3···O-1. The calculated rotations around the C-3—O-3 bond in the molecule of 1,6-anhydro-β-D-glucopyranose, per-*O*-acetyl-1,6-anhydro-β-D-glucopyranose, and in its derivative protonated in the position 3 point to the same fact (Fig. 1). In the most stable conformation of 1,6-anhydro-β-D-glucopyranose ($\varphi = 300°$) the hydrogen of C-3—OH is oriented towards the O-1 atom, enabling the formation of hydrogen bond which contributes to stabilization of this position. In per-*O*-acetylated 1,6-anhydro-β-D-glucopyranose the most stable conformation is the one ($\varphi = 60°$) where C-3—OAc is oriented outwards from the ring, the energy of the second conformer ($\varphi = 180°$) is higher by about 8.5 kJ mol^{-1}, while the acetyl group is oriented also outwards from the ring. The other positions are not advantageous because of steric reasons. Protonation of O-3 leads to a drastic change. Owing to steric interactions, the rotational freedom is very limited. The most stable is the conformer ($\varphi = 170°$) where the proton is oriented towards O-1 and stabilized

by the O-3···H···O-1 hydrogen bond. The energy of the nonprotonated form of the originally most stable conformer is higher by about 17.5 kJ mol^{-1}.

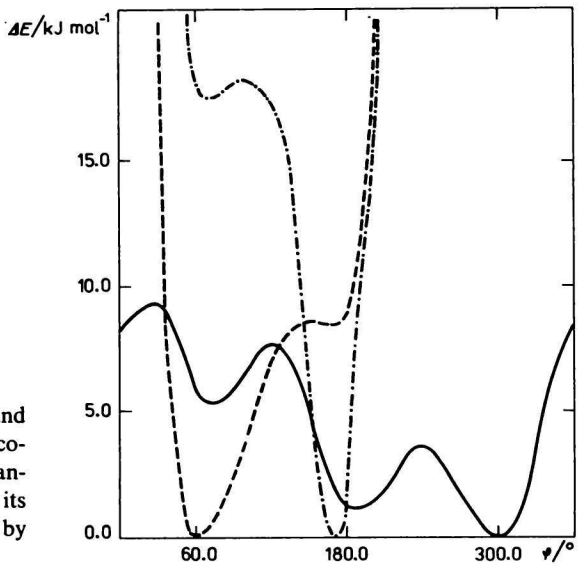


Fig. 1. Energetical rotation course around C-3—O-3 bond in 1,6-anhydro-β-D-glucopyranose (———), per-*O*-acetyl-1,6-anhydro-β-D-glucopyranose (– – – –), and its protonated derivative (–·–·–) calculated by PCILO quantum-chemical method.

The increased stability of the whole molecule of 2,3,4-tri-*O*-acetyl-β-D--glucopyranose as well as of the ester bond at C-3 to methanolysis in dependence on the length of the alkyl attached to the ester group is brought about, in addition to steric reasons, by the increasing positive inductive effect of the alkyl on the carbonyl carbon. In consequence of this the stability of the hydrogen bond between O-3 and O-1 increases and the nucleophilic addition of methanol in the second step becomes rather difficult. The calculated PCILO charges on the selected atoms around the reaction centre for C-3-substituted derivatives of 2,4-di-*O*-acetyl-1,6-anhydro-β-D-glucopyranose are presented in Table 4. The

Table 4

Influence of the size of substituents on PCILO charge distribution (in *e*) on the atoms selected in 3-*O*-substituted derivatives of 2,4-di-*O*-acetyl-1,6-anhydro-β-D-glucopyranose

Atom	$COCH_3$	COC_2H_5	COC_3H_7	COC_4H_9
O-1	−0.1652	−0.1666	−0.1666	−0.1666
HO-3	0.3047	0.3046	0.3044	0.3043
O-3	−0.0477	−0.0505	−0.0504	−0.0504
C(carboxyl)-3	0.3332	0.3246	0.3208	0.3207
O(carboxyl)-3	−0.1447	−0.1507	−0.1513	−0.1515

results show that with the increasing alkyl of the acyloxy group at C-3 the positive charge on carbon decreases and the negative charge on oxygen of the carbonyl group increases. The most significant change was observed in turning from acetyl to propionyl derivative. Further lengthening of the alkyl chain had no noticeable effect.

The expected course of methanolysis has been supported also by the results of esterification of 1,6-anhydro-β-D-glucopyranose. Due to the assumed hydrogen bond between O-3 and O-1 the first step of esterification (addition of acylpyridinium cation) of C-3—OH is faster than of C-2—OH and C-4—OH. However, the second step, *i.e.* splitting off of the proton is slower and, consequently, esterification of C-2—OH and C-4—OH proceeds preferentially. The increase in positive inductive effect of the substituent attached to the carbonyl carbon in the ester group is reflected in continuous increase of stability of the hydrogen bond between O-3 and O-1 and thus in increase of the yield of 2,4-di-*O*-acyl derivative (2,4-di-*O*-acetyl ≈ 15 % [3], 2,4-di-*O*-butyryl ≈ 24 %, 2,4-di-*O*-palmitoyl ≈ 34 %).

In hydrazinolysis the nucleophilic attack by the free electron pair of hydrazine nitrogen on the carbonyl carbon of ester takes place first. In the second step the hydrazide of carboxylic acid is split off under simultaneous addition of a proton and formation of C—OH. The hydrogen of the formed hydroxyl group at C-3 may then form a hydrogen bond with O-1 in consequence of which the rate of hydrazinolysis of the ester bond at C-3 is higher than at C-2 and C-4. This is in accordance with the results obtained. The increasing $+I$ effect of the substituent on the carbonyl carbon ($C_{15}H_{31}$, C_3H_7, CH_3) indirectly increases the electron density of oxygen in the ester linkage. In the case of the ester bond at C-3 (steric closeness of O-3 and O-1) it means a decrease in stability of this bond in comparison with those at C-2 and C-4. However, the results show that with lengthening of the alkyl chain in the ester group the selectivity of hydrazinolysis decreases (the forming 2,4-, 2,3-, and 3,4-di-*O*-acetyl, -butyryl, and -palmitoyl derivatives are in the ratio of 4 : 1 : 1, 2 : 1 : 1, and 1 : 1 : 1). We assume that in this case the steric factors (increase in bulkiness of the ester groups) dominate over the O-1···O-3 interactions.

Experimental

The following starting compounds were used: 1,6-anhydro-β-D-glucopyranose [4] (*I*): m.p. = 178—180 °C, [α] (D, $\varrho = 10\,\mathrm{g\,dm^{-3}}$, H_2O) = − 67.2°; 1,6-anhydro-2,3,4-tri-*O*-butyryl-β-D-glucopyranose [5] (*II*, sirup): [α] (D, $\varrho = 10\,\mathrm{g\,dm^{-3}}$, $CHCl_3$) = − 37.5°; 1,6-anhydro-2,3,4-tri-*O*-palmitoyl-β-D-glucopyranose [6] (*IX*): m.p. = 68—69 °C, [α] (D, $\varrho = 10\,\mathrm{g\,dm^{-3}}$, $CHCl_3$) = − 21.0°; 1,6-anhydro-2,4-di-*O*-benzyloxycarbonyl-β-D-glucopyranose [2] (*XVI*): m.p. = 122—123 °C, [α] (D, $\varrho = 10\,\mathrm{g\,dm^{-3}}$, $CHCl_3$) = − 36.4°.

Melting points were determined on a Kofler hot-stage, optical rotations were recorded with a Perkin—Elmer, model 141 polarimeter.

The PCILO semiempirical method [7], applied earlier in conformational studies of saccharides [8], was used for quantum-chemical calculations. Geometric parameters of the individual molecules were based on X-ray geometry of 1,6-anhydro-β-D-glucopyranose [9] and standard values of geometric parameters [10]. The proton position was optimized for the most stable conformers which were obtained by cyclic optimization of three torsional angles, while the other geometric parameters were constant.

Gas chromatography was performed on a Hewlett—Packard, model 5830 A chromatograph equipped with a flame ionization detector. A stainless-steel column (300 cm × 0.2 cm), packed with Supelcoport (0.135—0.150 mm grain size) coated with 3 % OV 225 stationary phase, was used. Heating of the column was programmed with a thermal gradient 1 °C min^{-1}, starting from 135 °C (6 min isotherm) to 220 °C (30 min isotherm). The injection port temperature was 220 °C, detector temperature 300 °C, carrier gas nitrogen at the flow rate of 15 cm^3 min^{-1}. The results were computed in normalized percentage after calibration with reference substances.

The course of hydrolysis in acid medium of methanolic hydrogen chloride was monitored by gas chromatography of samples (0.1 cm^3) withdrawn in certain time intervals. The liberated hydroxyl groups were converted into trifluoroacetyl derivatives by addition of trifluoroacetic anhydride (0.2 cm^3) to the aliquot withdrawn. The same procedure was applied in monitoring the alkaline hydrolysis with hydrazine hydrate. The hydrolysis was stopped by addition of acetone (0.1 cm^3). After evaporation of each sample, trifluoroacetic anhydride (0.2 cm^3) was added to the obtained residue.

^{1}H NMR spectra were recorded with a Bruker AM-300 spectrometer at the working frequency 300 MHz and 135 MHz in 5 mm cells; pulse width 2 μs, spectral width 3500 Hz, data points 32768 with relaxation delay 1 s at 297 K. The spectra were measured in acetone using TMS as internal standard. Chemical shifts were assigned by using homocorrelated 2D spectra (COSY-45). The ^{1}H NMR data of the individual derivatives are presented in Table 3.

Silufol sheets (Kavalier, Votice) were used for qualitative chromatographic separation of acyl derivatives, when monitoring the course of hydrolysis. The spots were visualized by spraying the sheets with 5 % H_2SO_4 in methanol and heating to 100 °C. Preparative chromatographic separation of the hydrolytic products was performed on a silica gel column (L-100/250, Lachema, Brno) by using the following elution systems: *A*: benzene—ethyl acetate ($\varphi = 2:1$), *B*: benzene—acetone ($\varphi = 9:1$), *C*: benzene—ethyl acetate—2-propanol ($\varphi = 8:4:1$), *D*: benzene—cyclohexane—ethyl acetate ($\varphi = 9:9:2$), *E*: benzene—hexane—ether ($\varphi = 1:1:15$), and *F*: chloroform—ethanol ($\varphi = 1:1$).

Acylation of 1,6-anhydro-β-D-glucopyranose (I) with butyric anhydride

The solution of butyric anhydride (6.3 g; 0.04 mol) in pyridine (10 cm^3) was added to the solution of *I* (1.62 g: 0.01 mol) in pyridine (50 cm^3) and the reaction mixture was allowed to stay at room temperature for 24 h. Then the mixture was poured into water (200 cm^3). The formed oil was extracted with $CHCl_3$ (2 × 100 cm^3) and the chloroform solution was washed successively with 0.5 M-HCl, 0.5 M-$NaHCO_3$, and water. After

drying and evaporation of the chloroform extract, a sirup (6.3 g) was obtained which, according to TLC determination in system *A*, contained tri-, di-, and monobutyryl derivatives of *I* in the mole ratio of 2 : 2 : 1. Preparative separation of the mixture on the silica gel column in system *A* afforded the following butyryl derivatives: 2,3,4-tri-*O*-butyryl (*II*, sirup): yield = 1.5 g, 39.6 %, $[\alpha]$ (D, $\varrho = 10\,\mathrm{g\,dm^{-3}}$, $CHCl_3$) = − 37.8°; a mixture of 2,4- and 2,3-di-*O*-butyryl (*III* and *IV*): yield = 1.4 g; 3,4-di-*O*-butyryl (*V*, sirup): yield = 0.42 g, 13.9 %, $[\alpha]$ (D, $\varrho = 10\,\mathrm{g\,dm^{-3}}$, $CHCl_3$) = 29.3°, for $C_{14}H_{22}O_7$ (M_r = 302.3) w_i(calc.): 55.61 % C, 7.35 % H; w_i(found): 55.60 % C, 7.38 % H; 4-mono-*O*-butyryl (*VII*): yield = 0.18 g, 7.7 %, m.p. = 42—44 °C, $[\alpha]$ (D, $\varrho = 10\,\mathrm{g\,dm^{-3}}$, $CHCl_3$) = − 71.1°, for $C_{10}H_{16}O_6$ (M_r = 232.2) w_i(calc.): 51.71 % C, 6.96 % H; w_i(found): 51.75 % C, 6.95 % H; 2-mono-*O*-butyryl (*VIII*, sirup): yield = 0.2 g, 8.6 %, $[\alpha]$ (D, $\varrho = 10\,\mathrm{g\,dm^{-3}}$, $CHCl_3$) = − 31.4°, for $C_{10}H_{16}O_6$ (M_r = 232.2) w_i(found): 51.73 % C, 6.95 % H. Rechromatography of the mixture of *III* and *IV* (1.4 g) in system *B* gave 2,3-di-*O*-butyryl (*III*, sirup): yield = 0.56 g, 18.5 %, $[\alpha]$ (D, $\varrho = 10\,\mathrm{g\,dm^{-3}}$, $CHCl_3$) = − 71.3°, for $C_{14}H_{22}O_7$ (M_r = 302.3) w_i(found): 55.60 % C, 7.34 % H; 2,4-di-*O*-butyryl (*IV*, sirup): yield = 0.72 g, 23.8 %, $[\alpha]$ (D, $\varrho = 10\,\mathrm{g\,dm^{-3}}$, $CHCl_3$) = − 54.1°, for $C_{14}H_{22}O_7$ (M_r = 302.3) w_i(found): 55.62 % C, 7.38 % H.

Hydrolysis of 1,6-anhydro-2,3,4-tri-O-butyryl-β-D-glucopyranose (II) with methanolic hydrogen chloride

2,3,4-Tri-*O*-butyryl derivative (*II*) (3.8 g) was dissolved in anhydrous methanol (32 cm^3) and 4 % methanolic hydrogen chloride (4 cm^3) was added. Then the reaction mixture was allowed to stay at 20 °C. The course of hydrolysis was monitored by TLC in systems *A* and *B* or by gas chromatography (Table 1). Hydrolysis on a preparative scale was stopped after 24 h by neutralization of the mixture with Amberlite IRA-402 (HCO_3). The ion exchanger was filtered off, the filtrate was concentrated and subjected to separation on the silica gel column in system *A*. The following derivatives were obtained: *II* (yield = 0.7 g, 18.5 %); *III* (yield = 0.75 g, 24.8 %) which, according to NMR spectrum, contained traces of *IV*; *V* (yield = 0.45 g, 14.9 %); *VI* (yield = 0.53 g, 22.8 %) containing also 5 % of *VII*.

Hydrolysis of II with hydrazine hydrate

To the solution of *II* (3.8 g) in pyridine (40 cm^3) 80 % hydrazine hydrate (0.76 cm^3) was added. The course of hydrolysis was monitored by TLC (systems *A*, *B*) and gas chromatography (Table 2). Hydrolysis on a preparative scale was stopped after 6 h by addition of acetone (20 cm^3). Concentration of the mixture afforded a sirup which was separated on the silica gel column in system *A* to give *II* (yield = 0.57 g, 15 %); a mixture of *III* and *IV* (yield = 1.5 g); *V* (yield = 0.32 g, 10.8 %). Rechromatography of the mixture in system *B* resulted in *III* (yield = 0.34 g, 11.4 %) and *IV* (yield = 0.62 g, 20.8 %).

Preparation of 1,6-anhydro-3-O-butyryl-β-D-glucopyranose (VI)

To the solution of 1,6-anhydro-2,4-di-*O*-benzyloxycarbonyl-β-D-glucopyranose (3 g) in pyridine (10 cm^3) butyric anhydride (5 g) was added under stirring and the mixture was kept at room temperature for 70 h. Then it was poured into water (100 cm^3) and the formed oil was extracted with $CHCl_3$ (2 × 50 cm^3). The chloroform extract was washed successively with 0.5 % HCl, 5 % $NaHCO_3$, H_2O, dried, and concentrated. The sirupy residue (5 g), containing mainly 1,6-anhydro-2,4-di-*O*-benzyloxycarbonyl-3-*O*-butyryl-β-D-glucopyranose contaminated with butyric acid, was dissolved in tetrahydrofuran (60 cm^3). Then Pd/C (2.5 g) was added and the mixture was saturated with H_2. The reaction course was monitored by TLC in system *A*. After completion of hydrogenation (≈ 10 h) the catalyst was filtered off, the filtrate was concentrated and the distillation residue was extracted with H_2O (50 cm^3, 80 °C). Concentration of the water extract gave *VI*: yield = 0.7 g, 43.7 %, m.p. = 63—67 °C. Recrystallization from the mixture of ethyl acetate—petroleum ether afforded a compound of m.p. = 65—68 °C, [α] (D, $\varrho = 10\,g\,dm^{-3}$, $CHCl_3$) = −64.2°, for $C_{10}H_{16}O_6$ ($M_r = 232.2$) w_i(found): 51.70 % C, 6.91 % H.

Acylation of 1,6-anhydro-β-D-glucopyranose (I) with palmitic anhydride

a) Preparation of di-O-palmitoyl derivatives

To the solution of *I* (0.81 g; 5 mmol) in pyridine (10 cm^3) palmitic anhydride (9.8 g; 0.02 mol) in pyridine (90 cm^3) was added and the mixture was kept at room temperature for 72 h. Then it was poured into water (500 cm^3), the precipitate was filtered off and dried. The obtained product (8.5 g) was composed, according to TLC in system *D*, of 30 % 2,3,4-tri-*O*-palmitoyl derivative (*IX*), 55 % di-*O*-palmitoyl derivatives, 15 % mono-*O*-palmitoyl derivatives, and palmitic acid. Preparative column separation in system *D* afforded the following fractions: *IX*: yield = 1.18 g, 27 %; 3,4-di-*O*-palmitoyl derivative (*X*): yield = 0.14 g, 4.5 %, m.p. = 53—54 °C (CH_3OH), [α] (D, $\varrho = 10\,g\,dm^{-3}$, $CHCl_3$) = −33.8°, for $C_{38}H_{70}O_7$ ($M_r = 639.08$) w_i(calc.): 71.41 % C, 11.06 % H; w_i(found): 71.45 % C, 11.02 % H; 2,3-di-*O*-palmitoyl derivative (*XI*): yield = 0.31 g, 9.8 %, m.p. = 65—66 °C (CH_3OH), [α] (D, $\varrho = 10\,g\,dm^{-3}$, $CHCl_3$) = −9.9°, for $C_{38}H_{70}O_7$ ($M_r = 639.08$) w_i(found): 71.40 % C, 11.03 % H; 2,4-di-*O*-palmitoyl derivative (*XII*): yield = 1.08 g, 34 %, m.p. = 85—86 °C (CH_3OH), [α] (D, $\varrho = 10\,g\,dm^{-3}$, $CHCl_3$) = −29.1°, for $C_{38}H_{70}O_7$ ($M_r = 639.08$) w_i(found): 71.78 % C, 11.01 % H.

b) Preparation of mono-O-palmitoyl derivatives

To the solution of *I* (0.81 g; 5 mmol) in pyridine (10 cm^3) palmitic anhydride (4.9 g; 10 mmol) was added and the reaction mixture was kept at room temperature for 72 h. Then it was poured into water, the precipitate was filtered off and dried. The obtained mixture (4.2 g) contained, according to TLC in systems *D* and *E*, mainly monopalmitoyl

derivatives contaminated with palmitic acid. Column chromatographic separation in system *E* gave 4-*O*-palmitoyl derivative (*XIII*): yield = 0.2 g, 5 %, m.p. = 73—74 °C (acetone—hexane), $[\alpha]$ (D, $\varrho = 5\ \mathrm{g\,dm^{-3}}$, $CHCl_3$) = −39.1°, for $C_{22}H_{40}O_6$ ($M_r = 400.43$) w_i(calc.): 65.95 % C, 10.08 % H; w_i(found): 65.98 % C, 10.05 % H; 2-*O*-palmitoyl derivative (*XV*): yield = 1.05 g, 26.2 %, m.p. = 64—66 °C (acetone—hexane), $[\alpha]$ (D, $\varrho = 10\ \mathrm{g\,dm^{-3}}$, $CHCl_3$) = −16.8°, for $C_{22}H_{40}O_6$ ($M_r = 400.43$) w_i(found): 65.96 % C, 10.05 % H.

Hydrolysis of 1,6-anhydro-2,3,4-tri-O-palmitoyl-β-D-glucopyranose (IX) with methanolic hydrogen chloride

To the solution of *IX* (5 g) in the mixture of $CHCl_3$—CH_3OH (400 cm^3, $\varphi = 1:1$) 4 % methanolic hydrogen chloride (200 cm^3) was added. Hydrolysis was stopped after 48 h by neutralization of the mixture with $BaCO_3$. After filtration and concentration a sirup (4.5 g) was obtained which contained, according to TLC in systems *D* and *E*, 50 % *IX*, 30 % di-*O*-palmitoyl and 20 % mono-*O*-palmitoyl derivatives. Preparative column separation in system *D* afforded, in addition to the starting 2,3,4-tri-*O*-palmitoyl derivative *IX*, 3,4-di-*O*-palmitoyl derivative (*X*): yield = 0.62 g, 17 %, m.p. = 53 °C, $[\alpha]$ (D, $\varrho = 10\ \mathrm{g\,dm^{-3}}$, $CHCl_3$) = −33.6°; 2,3-di-*O*-palmitoyl derivative (*XI*): yield = 0.24 g, 6.6 %, m.p. = 65—66 °C, $[\alpha]$ (D, $\varrho = 5\ \mathrm{g\,dm^{-3}}$, $CHCl_3$) = −10.1°; 2,4-di-*O*-palmitoyl derivative (*XII*): yield = 0.1 g, 2.7 %, m.p. = 85—86 °C, $[\alpha]$ (D, $\varrho = 5\ \mathrm{g\,dm^{-3}}$, $CHCl_3$) = −29.5°. Then the column was eluted with system *E* to give a mixture of mono-*O*-palmitoyl derivatives (0.5 g) which, according to TLC in system *E*, was composed of 20 % *XIII*, 65 % *XIV*, and 15 % *XV*.

1,6-Anhydro-3-O-palmitoyl-β-D-glucopyranose (XIV)

Palmitic anhydride (5 g) in pyridine (10 cm^3) was added at room temperature to the solution of 1,6-anhydro-2,4-bis-*O*-benzyloxycarbonyl-β-D-glucopyranose (1.6 g) in pyridine (10 cm^3) and the reaction mixture was heated at 70 °C for 5 h. After cooling it was poured into water (200 cm^3), the precipitate was filtered off, dried, and fractionated on the silica gel column in system *B*. The fraction containing 1,6-anhydro-2,4-di-*O*-benzyloxycarbonyl-3-*O*-palmitoyl-β-D-glucopyranose (1.3 g) was dissolved in tetrahydrofuran (10 cm^3). Then 5 % Pd/C (1 g) was added and the mixture was hydrogenated at room temperature and atmospheric pressure. After completion of hydrogenolysis ($\approx$ 10 h) the catalyst was filtered off and the filtrate was concentrated to give the product *XIV*: yield = 0.4 g, 51.9 %, m.p. = 68—69 °C (acetone), $[\alpha]$ (D, $\varrho = 10\ \mathrm{g\,dm^{-3}}$, $CHCl_3$) = −37.2°, for $C_{22}H_{40}O_6$ ($M_r = 4000.43$) w_i(found): 65.91 % C, 10.02 % H.

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