PARTIALLY ACETYLATED SUCROSE. STRUCTURES OF HEPTA-O-ACETYLSUCROSES FORMED BY DEACETYLATION OF OCTA-O-ACETYLSUCROSE

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Dedicated to Prof. Jiří Jarý on the occasion of his 60th birthday.

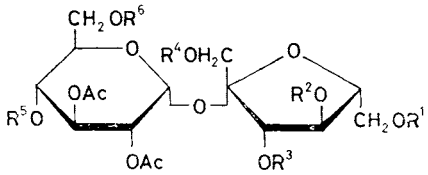
Formation of hepta-O-acetylsucroses *I*, *II*, *XI*, and *XII*, having a free hydroxyl group on the fructose moiety, in deacetylation of octa-O-acetylsucrose on aluminum oxide impregnated by potassium carbonate was proved by preparative chromatography on silica gel and by liquid chromatography. Their structures were inferred from the ^{1}H and ^{13}C NMR spectra, from NMR and mass spectra of their mono-methanesulfonyl derivatives *VI*—*IX*, or using comparison with authentic samples. Methanolic solutions of hepta-O-acetylsucroses *I* and *II*, a mixture of hepta-O-acetylsucroses *XI* and *XII*, and octa-O-acetylsucrose were deacetylated on aluminum oxide impregnated by potassium carbonate and the time dependence of the reaction mixture composition was studied by liquid chromatography. Kinetic measurements show that deacetylation is first order reaction with respect to the starting compounds. With octa-O-acetylsucrose, the most reactive group is that at position 4′, so that formation of 1′,2,3,3′,4,6,6′-hepta-O-acetylsucrose (*II*) is preferred. Both this compound and 1′,2,3,4,4′,6,6′-hepta-O-acetylsucrose (*XII*) are deacetylated to 1′,2,3,4,6,6′-hexa-O-acetylsucrose (*V*). From 2,3,3′,4,4′,6,6′-hepta-O-acetylsucrose (*XI*), the 2,3,4,4′,6,6′-hexa-O-acetylsucrose (*IV*) results. Other hexa-O-acetylsucroses, formed by deacetylation of hepta-O-acetylsucroses *I* and *II*, are deacetylated to penta-O-acetylsucroses faster than the hexa-O-acetylsucrose *V* and their proportion in the mixture after deacetylation of octa-O-acetylsucrose is very low. No migration of the O-acetyl group from the position 4′ to the position 6′ or *vice versa* occurs during the deacetylation.

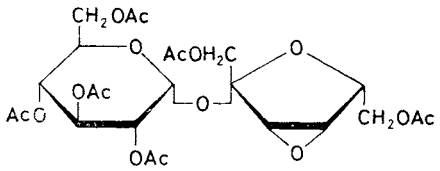
Ballard and coworkers[1,2] have found that a mixture of four hepta-O-acetylsucroses resulted from deacetylation of chloroform solution of octa-O-acetylsucrose on the aluminum oxide column. Among them they identified the prevailing 1′,2,3,3′,4,4′,6-hepta-O-acetylsucrose (*I*), and further 1′,2,3,3′,4,6,6′-hepta-O-acetylsucrose (*II*) and 1′,2,3,3′,4′,6,6′-hepta-O-acetylsucrose (*III*). These authors assumed that the deacetylation of octa-O-acetylsucrose takes place preferentially at positions 6 and 6′ so that the hepta-O-acetylsucoses *II* and *III* are products of the O-acetyl group migra-

tion from the position 4 to the position 6, or from the position 4' to the position 6', respectively. We have observed that deacetylation of methanolic solution of octa-O--acetylsucrose on aluminum oxide impregnated by potassium carbonate[3] gave besides the hepta-O-acetylsucroses in 34% yield the mixture of 2,3,4,4',6,6'-hexa-O-acetylsucrose (*IV*) and 1',2,3,4,6,6'-hexa-O-acetylsucrose (*V*) with the later component strongly prevailing[4]. We wondered whether the preponderant hexa-O-acetylsucrose *V* is indeed formed by deacetylation of octa-O-acetylsucrose in positions 3' and 4' or whether there is O-acetyl group migration. For this reason and also to compare our method of octa-O-acetylsucrose deacetylation with that already reported[1], we deal here with the analysis of the mixture of hepta-O-acetylsucroses obtained by our method[3].

Chromatographic fraction from the reaction mixture after deacetylation of octa--O-acetylsucrose on impregnated aluminum oxide[4], corresponding according to the thin-layer chromatography with flame-ionization detection to hepta-O-acetylsucroses[5], was separated by repeated chromatography on silica gel[2]. Three fractions, homogeneous on thin-layer chromatography, were obtained in ratio 15 : 40 : 45. The first one was crystalline and identical to hepta-O-acetylsucrose *I* (refs[1,5-7]). The prevailing third fraction was according to ^{1}H and ^{13}C NMR spectra identical with hepta-O-acetylsucrose *II* (ref.[2]). Some signals in the ^{13}C NMR spectrum of the second fraction were doubled: C-1 (89·6 and 89·9 ppm), C-2' (103·9 and 105·2 ppm), and C-5' (77·6 and 78·9 ppm). Chemical shifts of glucose carbons were very similar for both compounds and close to that of corresponding carbon atoms in the spectrum of octa--O-acetylsucrose. That indicated a mixture of two hepta-O-acetylsucroses with hydroxyl groups located on the fructose moiety. Two new signals appeared in the ^{1}H NMR spectrum upon addition of trichloroacetyl isocyanate (TAI)[8-10] at 8·98 and 9·50 ppm, confirming again the two-component mixture. The reaction of this mixture with methanesulfonyl chloride in pyridine gave a mixture of two compounds *VI* and *VII* that were separated by silica gel chromatography. For the purpose of comparison, we prepared the corresponding methanesulfonyl derivatives from the hepta-O-acetylsucroses *I* and *II*: 1',2,3,3',4,4',6-hepta-O-acetyl-6'-O-methanesulfonylsucrose (*VIII*) and 1',2,3,3',4,6,6'-hepta-O-acetyl-4'-O-methanesulfonylsucrose (*IX*). There are ions *m/z* 331 and 367 in the mass spectra of all four methanesulfonyl derivatives *VI*–*IX*. The later one is always of higher intensity. That shows that the mesyloxy group is in all cases located on the fructofuranose part[2,11]. Low-field parts of the ^{1}H NMR spectra of compounds *VI*–*IX* differ in details but do not permit safe conclusions concerning the location of the mesyloxy groups. On the contrary, all ^{13}C NMR spectra of compounds *VI*–*IX* were clearly different. Chemical shifts of carbon atoms C-1 to C-6 are very similar and resemble the values of octa-O--acetylsucrose. That supports the conclusion based on the the mass spectra that mesyloxy group in compounds *VI*–*IX* is located on the fructose moiety. From the electronegativity difference between the mesyloxy and acetoxy groups it can be ex-

pected that the resonance of the carbon carrying the mesyloxy group will be shifted downfield with respect to that carrying the acetoxyl; the neighbour carbons should then resonate upfield. Methanesulfonyl derivative *VI* has similarly to 6'-O-methanesulfonyl derivative *VIII* one methyleneoxy carbon atom shifted 5·1 ppm downfield and therefore posseses the structure of 2,3,3',4,4',6,6'-hepta-O-acetyl-1'-O-methanesulfonylsucrose (*VI*). The 1·0 ppm upfield shift of C-2' agrees well with this conclusion. Methanesulfonyl derivative *VII* differs from the 4'-O-methanesulfonyl derivative *IX*. Compared with octa-O-acetylsucrose, C-3' in compound *VII* resonates 2·5 ppm downfield and both signals of C-2' and C-4' are shifted 1·3 ppm upfield. From this follows that compound *VII* is 1',2,3,4,4',6,6'-hepta-O-acetyl-3'-O-methanesulfonylsucrose. The structure of compound *IX* was further confirmed by its transformation to 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl-1,6-di-O-acetyl-3,4-anhydro-β-D-lyxohexulofuranoside (*X*) (ref. [12]).





X

I, R^1= H; R^2= R^3= R^4= R^5= R^6= Ac
II, R^2= H; R^1= R^3= R^4= R^5= R^6= Ac
III, R^5= H; R^1= R^2= R^3= R^4= R^6= Ac
IV, R^3= R^4= H; R^1= R^2= R^5= R^6= Ac
V, R^2= R^3= H; R^1= R^4= R^5= R^6= Ac
VI, R^4= Ms; R^1= R^2= R^3= R^5= R^6= Ac
VII, R^3= Ms; R^1= R^2= R^4= R^5= R^6= Ac
VIII, R^1= Ms; R^2= R^3= R^4= R^5= R^6= Ac
IX, R^2= Ms; R^1= R^3= R^4= R^5= R^6= Ac
XI, R^4= H; R^1= R^2= R^3= R^5= R^6= Ac
XII, R^3= H; R^1= R^2= R^4= R^5= R^6= Ac
XIII, R^5= R^6= H; R^1= R^2= R^3= R^4= Ac
XIV, R^5= Ms; R^1= R^2= R^3= R^4= R^6= Ac

In formulae *I* - *XIV* Ms methansulfonyl, Ac acetyl.

From the above given results it follows that using our procedure[3], octa-O-acetylsucrose is deacetylated exclusively on the fructose moiety, yielding hepta-O-acetylsucroses *I*, *II*, *XI*, and *XII*. However, when the method of British authors[1,2] is used, octa-O-acetylsucrose also provides the hepta-O-acetylsucrose *III* with free hydroxyl group in the glucose part of the molecule. Therefore, we synthetized this compound and examined its chromatographical behaviour. Compound *III* was prepared by partial acetylation of easily available 1',2,3,3',4',6'-hexa-O-acetylsucrose (*XIII*)[13] and further characterized by its transformation to crystalline 4-O-methanesulfonyl derivative *XIV* (refs[14-16]). The R_F value of compound *III* in thin-layer chromato-

graphy on silica gel was clearly different from the R_F values of the hepta-O-acetylsucroses *I*, *II*, *XI*, and *XII*. Compound *III* was not detected either in the reaction mixture from the deacetylation of octa-O-acetylsucrose nor in the studied mixture of hepta-O-acetylsucroses.

We tried to solve the question of possible O-acetyl group migration during deacetylation reaction by kinetic study of deacetylation of octa-O-acetylsucrose and hepta-O-acetylsucroses, respectively. The requirement of quantitative analysis of O-acetylsucroses with degree of acetylation 5–8 in the mixture of all sucrose acetates (acetylation degree 1–8) without cumbersome sample preparation was best met by reverse phase liquid chromatography. Hepta-O-acetylsucroses *XI* and *XII*, that were not resolved on silica gel thin-layer chromatography, showed good separation under the conditions used. In one chromatographic fraction from the separation of the hepta-O-acetylsucroses mixture, the proportions of compounds *XI* and *XII* were unequal (*XI* : *XII*, 2 : 1). ^{13}C NMR spectrum of this mixture showed that C-2′ of the prevailing isomer resonated at 105·2 ppm. Therefore, it is assigned to compound *XI* having a hydroxyl group at C-1′ (β-effect due to the replacement of an acetoxy group by a hydroxyl one). The chromatographic peak corresponding to the compound with lowest capacity factor among hepta-O-acetylsucroses (Fig. 1) is therefore due to compound *XI*. Contrary to silica gel thin-layer chomatography, where compound *I* is well resolved from the mixture of *XI* and *XII*, are the compounds *I* and *XII* not separable by reverse phase liquid chromatography. Compound *II* is sufficiently different from the other hepta-O-acetylsucroses on reverse phase chromatography. Hexa-O-acetylsucroses *IV* and *V* are also separated in this experiment. Methyl acetate formed during deacetylation exhibit a capacity factor falling

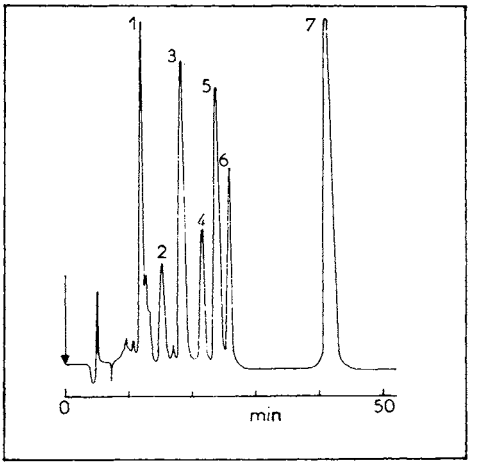


FIG. 1

Chromatographic analysis of mixture of O-acetylsucroses with different degree of acetylation (for details see Experimental). 1 methyl acetate and penta-O-acetylsucroses, 2 hexa-O-acetylsucrose *IV*, 3 hexa-O-acetylsucrose *V*, 4 hepta-O-acetylsucrose *XI*, 5 hepta-O-acetylsucrose *II*, 6 hepta-O-acetylsucroses *I* and *XII*, 7 octa-O-acetylsucrose

in the range of penta-O-acetylsucroses. To avoid the interference, the samples were evaporated to dryness prior to analysis. Kinetic measurements were performed as already described[4], *i.e.* under conditions of pseudomonomolecular reaction (roughly 200-fold excess of methanol to one acetoxy group of octa-O-acetylsucrose). We studied the deacetylation of octa-O-acetylsucrose, hepta-O-acetylsucroses *I* and *II* as well as that of mixture of *XI* and *XII* in the ratio 2 : 1. The content of O-acetylsucroses with acetylation degree 5–8 was determined.

The results of octa-O-acetylsucrose deacetylation are shown in Table I and Fig. 2. Time dependence of the logarithm of octa-O-acetylsucrose concentration is linear (correlation coefficient 0·992). Therefore, the deacetylation of octa-O-acetylsucrose is a first order reaction with respect to the starting compound with the rate constant $k = 3{\cdot}583 \cdot 10^{-5}\ s^{-1}$. The reaction course correspond to a system of consecutive reactions in which some steps can even involve concurent reactions. The fastest reaction is formation of hepta-O-acetylsucrose *II* with hydroxyl group at the position 4'. This compounds also prevails (43%) in the mixture of hepta-O-acetylsucroses after completing the reaction; there is 23% of hepta-O-acetylsucrose *XI* and the

TABLE I

Time dependence of the reaction mixture composition in deacetylation of methanolic octa-O-acetylsucrose on aluminum oxide impregnated by potassium carbonate

Reaction time h	Concentration, mass %						
	Octa-O-acetylsucrose	*I* + *XII*	*II*	*XI*	*V*	*IV*[a]	Penta-O-acetylsucrose
1	78·24	6·00	8·99	3·94	1·74	0·95	0·15
2	69·93	7·74	11·53	5·29	3·63	1·58	0·32
3	60·77	9·30	13·57	6·20	6·78	2·80	0·59
4	51·85	10·76	15·28	7·29	9·75	4·00	1·05
5	48·98	10·54	15·00	7·33	11·95	4·68	1·52
6	43·63	11·33	15·65	7·62	14·24	5·51	2·00
7	41·06	10·58	14·77	7·30	16·33	6·06	3·89
8	38·41	10·38	14·43	7·29	18·32	6·64	4·54
9	33·07	10·81	14·92	7·42	21·80	6·61	5·36
10	28·18	9·99	13·32	6·89	25·20	7·73	8·68
11	25·00	10·40	13·15	6·78	26·18	8·23	10·26
12	25·12	10·16	13·27	7·12	25·83	8·47	10·02
21[b]	16·48	7·59	9·59	5·19	33·77	8·59	18·78

[a] Together with hexa-O-acetylsucrose(s) of undetermined structure; [b] starting from the 13th hour, the mixture was left standing at room temperature.

mixture of *I* and *XII* accounts for 34%. Since we have isolated the compound *I* in 15% yield by preparative chromatography, the portion of compound *XII* is estimated as 19% of the hepta-O-acetylsucroses fraction. The time dependence of the concentrations of formed hexa-O-acetylsucroses *IV* and *V* is practically linear in first 10 h. The ratio *IV* : *V* is 1 : 4 after the reaction is completed; the initial ratio was 1 : 2. Hexa-O-acetylsucroses *IV* and *V* represent 42·5% of the mixture of the O-acetylsucroses with degree of acetylation 5–8; octa-O-acetylsucrose 16·5%, hepta-O-acetylsucroses 22·3%, and penta-O-acetylsucroses 18·7%. Taking the heterogeneous nature of the reaction into account, the agreement of these results with the preparative separation of the reaction mixture from a run made in more than 20 times larger scale is reasonably good (16·4% of octa-O-acetylsucrose, 18% of hepta--O-acetylsucroses, 41·4% of hexa-O-acetylsucroses, and 21% of penta-O-acetylsucroses)[4].

Following the deacetylation of hepta-O-acetylsucrose *II* (Fig. 3), we did not detect the presence of hepta-O-acetylsucrose *I* in the reaction mixture. Thus, no acetyl group migration from the position 6′ to the position 4′ takes place. Deacetylation is the first order reaction with respect to the starting compound *II*. We calculated the rate constant $k = 4{\cdot}222 \,.\, 10^{-5}\ s^{-1}$ from the time dependence of the logarithm of *II* concentration (correlation coefficient 0·997). Deacetylation of compound *II* gives hexa-O-acetylsucrose *V* and an another hexa-O-acetylsucrose with capacity

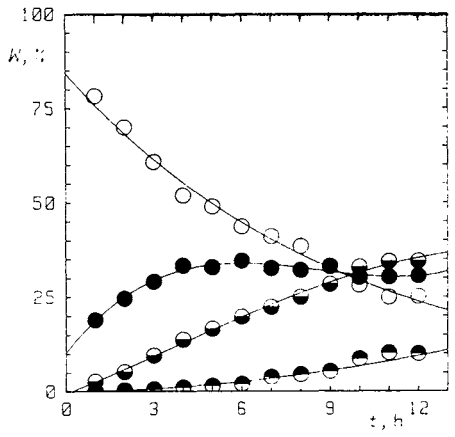


FIG. 2

Deacetylation of octa-O-acetylsucrose. Time dependence of concentration of octa-O--acetylsucrose ○, hepta-O-acetylsucroses ●, hexa-O-acetylsucroses ◒, and penta-O-acetylsucroses ◓

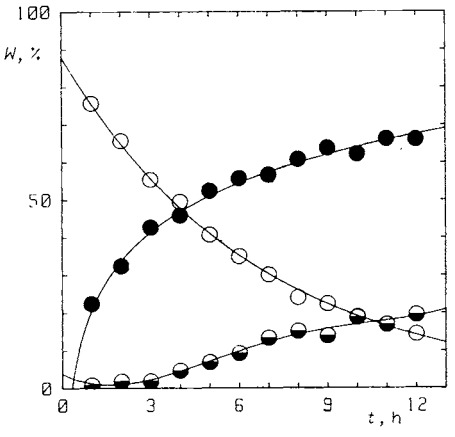


FIG. 3

Deacetylation of hepta-O-acetylsucrose *II*. Time dependence of concentration of hepta--O-acetylsucrose *II* ○, hexa-O-acetylsucrose *V* ●, and penta-O-acetylsucroses ◒

factor identical to that of hexa-O-acetylsucrose *IV*. The ratio of this minor component to compound *V* is 1 : 20 by the end of reaction. Considering the deacetylation of *II* as a system of consecutive reactions A → B → C with rate constants k_1 and k_2, respectively, it is possible to estimate from the relation (*1*)

$$[B]_t = [A]_{t=0} \frac{k_1}{k_2 - k_1} (e^{-k_1 t} - e^{-k_2 t}) \qquad (1)$$

that the subsequent deacetylation of hexa-O-acetylsucrose(s) is about five times slower than the deacetylation of hepta-O-acetylsucrose *II*.

Deacetylation of hepta-O-acetylsucrose *I* (Fig. 4) is also a first order reaction with respect to the starting compound. The rate constant $k = 3{\cdot}428 \cdot 10^{-5}\ s^{-1}$, (correlation coefficient 0·989). No hepta-O-acetylsucrose *II* is formed during the deacetylation. Therefore, the O-acetyl group migration from the position 4′ to the position 6′ is excluded. One hexa-O-acetylsucrose with capacity factor identical to that of compound *IV* is formed. Its deacetylation to penta-O-acetylsucrose(s) is about 2·5 times slower than that of hepta-O-acetylsucrose *I* but two times faster than the deacetylation of hexa-O-acetylsucrose *V*. Deacetylation of the mixture of hepta-O-acetylsucroses *XI* and *XII* in the ratio 2 : 1 (Fig. 5) is also a first order reaction with respect to the starting compounds. The correlation coefficients were

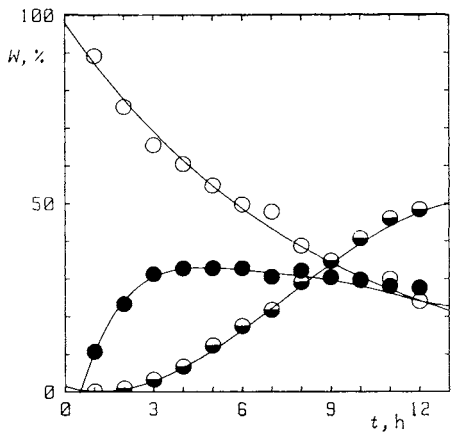


FIG. 4

Deacetylation of hepta-O-acetylsucrose *I*. Time dependence of concentration of hepta-O-acetylsucrose *I* ○, hexa-O-acetylsucrose of unknown structure ●, and penta-O-acetylsucroses ◒

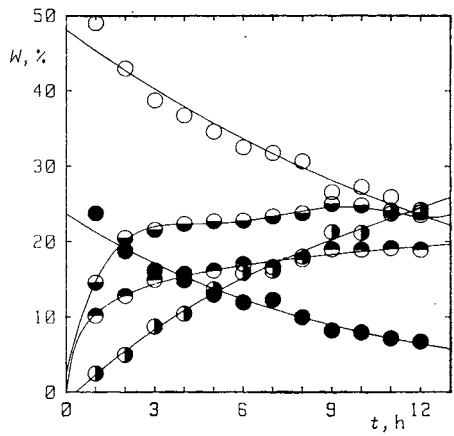


FIG. 5

Deacetylation of mixture of hepta-O-acetylsucroses *XI* and *XII*. Time dependence of concentration of hepta-O-acetylsucrose *XI* ○, *XII* ●, hexa-O-acetylsucroses *IV* ◒ and *V* ◓, and penta-O-acetylsucroses ◑

0·961 (*XI*) and 0·963 (*XII*) rate constants $1·611 . 10^{-5}$ s^{-1} (*XI*) and $2·944 . 10^{-5}$ s^{-1} (*XII*), respectively. Two hexa-O-acetylsucroses with the same capacity factors as compounds *IV* and *V* are formed.

From the above given results, it can be concluded that deacetylation of methanolic solution of octa-O-acetylsucrose on aluminum oxide impregnated by potassium carbonate is a transesterification reaction. Octa-O-acetylsucrose is deacetylated exclusively in the fructose moiety. The most reactive site is the position 4'. The major product has the structure *II*, other compounds are *I*, *XI*, and *XII*. These hepta-O-acetylsucroses are deacetylated to hexa-O-acetylsucroses without O-acetyl group migration from the position 4' to the position 6' and *vice versa*. The rate of this subsequent deacetylation decreases in order *II* > *I* > *XII* > *XI*. Hepta-O--acetylsucrose *II* is evidently deacetylated preferably at the position 3' and compound *XII* at the position 4', both giving the same final product – the hexa-O-acetylsucrose *V*. Minor hexa-O-acetylsucrose *IV* that we have identified in the reaction mixture[4] is evidently formed by deacetylation of the hepta-O-acetylsucrose *XI* at the position 3'. Besides these products, the hexa-O-acetylsucroses having the same capacity factor as the compound *IV* but probably a different structure, are formed from the hepta-O-acetylsucroses *I* and *II*. These compounds are deacetylated to penta-O--acetylsucroses faster than hexa-O-acetylsucrose *V* so that their concentration in the final mixture is very low.

EXPERIMENTAL

Melting points were determined on a Kofler apparatus and were not corrected. Optical rotations were measured on a Opton instrument in 20 cm cuvettes at 20°C using concentration $c = 1·0 \pm 0·3$. Thin-layer chromatography (TLC) was performed on silica gel according to Stahl (Merck, Darmstadt); particle size 10–40 μm, plates 25 × 75 mm, layer thickness 0·2–0·3 mm. Detection of compounds was achieved by spraying with 1% cerium (*IV*) sulfate in 10% sulfuric acid followed by heating. Liquid chromatography was performed on a glass column CGC 6802 3·2 × 150 mm, loaded by Separon Six C_{18}, particle size 5 μm at 25°C. Mobile phase methanol–water 55 : 45 v/v was used with flow rate 5 ml h^{-1}. UV detector (Pye Unicam PU 4020) was operated at 210 nm. When not stated otherwise, the preparative chromatography was performed on a silica gel column with particle size 100–160 μm (Lachema, Brno). The solvents were removed *in vacuo*, the bath temperature did not exceed 50°C. ^1H and ^{13}C NMR spectra were measured on a FT NMR spectrometer Jeol FX-60 with observing frequency 59·797 MHz for ^1H and 15·036 MHz for ^{13}C. The spectra were mostly measured in deuteriochloroform with tetramethylsilane as an internal standard. Chemical shifts are given in the δ-scale with accuracy $\pm 0·005$ ppm and $\pm 0·06$ ppm for ^1H and ^{13}C NMR spectra, respectively. Signal assignment is based on homonuclear decoupling and signal multiplicity (^1H NMR), on off-resonance, noise off-resonance, and selective heteronuclear decoupling experiments (^{13}C NMR). Mass spectra were recorded on a Jeol JMS DX-200 instrument equipped with a JMA 2000 computer.

Isolation of Hepta-O-acetylsucroses *I*, *II*, *XI*, and *XII*

Mixture of hepta-O-acetylsucroses (9·2 g) obtained from the reaction mixture after deacetylation

of octa-O-acetylsucrose on aluminum oxide impregnated by potassium carbonate[4] was subjected to chromatography on silica gel column (350 g, particle size 40—100 μm, eluent chloroform and chloroform–ethanol 100 : 1, respectively). Three chromatographic portions, TLC pure (chloroform–ethanol 100 : 1, three times developed) were obtained after re-chromatography of the mixed fractions. The first fraction (1·4 g, 15%), m.p. 146—160°C, after recrystallization from ethyl acetate–light petroleum had m.p. 158·5—160°C, $[\alpha]_D$ +53·5° (chloroform). Literature[7] gives for compound *I* m.p. 160°C, $[\alpha]_D$ +49·5° (chloroform), ref.[1] gives m.p. 160°C, $[\alpha]_D$ +52·5° (chloroform). ^{1}H NMR spectrum was identical with that published[1]. ^{13}C NMR spectrum: 20·7 q (7 C), 61·2 t, 61·5 t, 63·4 t, 68·0 d, 68·7 d, 69·4 d, 70·3 d, 73·7 d, 76·2 d, 81·6 d, 90·1 d, 103·3 s, 169·5 s, 170·0 s (4 C), 170·6 s (2 C). The second chromatographic fraction (3·7 g, 40%) was according to liquid chromatography and NMR spectroscopy a mixture of compounds *XI* and *XII*. ^{13}C NMR spectrum: 20·7 q, 61·8 t, 62·2 t, 63·2 t, 63·5 t, 63·7 t, 68·2 d, 68·4 d, 68·6 d, 69·7 d, 70·0 d, 70·2 d, 74·8 d, 76·4 d, 77·6 d, 78·8 d, 89·6 d, 89·9 d, 103·9 s, 105·2 s, 169·5 s, 170·0 s, 170·2 s, 170·7 s. The third fraction (4·1 g, 45%) has optical rotation $[\alpha]_D$ +53·2° (chloroform); ref.[2] gives for compound *II* $[\alpha]_D$ +54·3° (chloroform). ^{1}H NMR data were identical with that reported[2]. ^{13}C NMR spectrum: 20·7 q (7 C), 62·4 t, 63·4 t, 64·1 t, 68·5 d, 68·7 d, 69·9 d, 70·2 d, 73·3 d, 78·6 d, 80·4 d, 89·1 d, 102·8 s, 169·6 s, 170·1 s (2 C), 170·2 s, 170·8 s, 171·2 s, 171·3 s.

2,3,3′,4,4′,6,6′-Hepta-O-acetyl-1′-O-methanesulfonylsucrose (*VI*)
and 1′,2,3,4,4′,6,6′-Hepta-O-acetyl-3′-O-methanesulfonylsucrose (*VII*)

Methanesulfonyl chloride (0·2 ml) was added at −70°C to the 1 : 1 mixture of compounds *XI* and *XII* (309 mg) in pyridine (4 ml). The mixture was kept at −15°C overnight, decomposed by water and diluted by chloroform. The chloroform layer was gradually washed by cold 10% sulfuric acid, water, 5% sodium hydrogen carbonate, and water. The extract was dried over magnesium sulfate and the solvent was removed. The sirupy residue (379 mg) contained two compounds with close R_F values (around 0·6) according to TLC in the system benzene–acetone 8 : 2. Silica gel column chromatography (50 g) in the system benzene–acetone 95 : 5 provided 124 mg of compound *VI*, 150 mg of the mixture *VI* plus *VII* and 105 mg of *VII*.

Compound *VI* is amorphous and has $[\alpha]_D$ +45·5° (chloroform). For $C_{27}H_{38}O_{20}S$ (714·6) was calculated: 45·37% C, 5·36% H, 4·49% S; found: 45·06% C, 5·52% H, 4·59% S. ^{1}H NMR spectrum: 2·04 s (3 H), 2·10 s (15 H), 2·18 s (3 H), 3·12 s (3 H), 4·87 dd ($J_{1,2}$ = 3·7 Hz, $J_{2,3}$ = = 9·8 Hz, H-2), 5·06 t ($J_{3,4} = J_{4,5}$ = 9·8 Hz, H-4), 5·47 mt (3 H, H-3, H-3′, H-4′), 5·71 d ($J_{1,2}$ = 3·7 Hz, H-1). ^{13}C NMR spectrum: 20·5 q (7 C), 37·7 q, 61·7 t, 63·1 t, 67·5 t, 68·2 d, 68·7 d, 69·6 d, 70·1 d, 74·0 d, 75·5 d, 78·9 d, 89·8 d, 102·8 s, 169·3 s, 169·7 s (2 C), 169·9 s (2 C), 170·3 s, 170·6 s.

Compound *VII* was amorphous, too; $[\alpha]_D$ + 52·0° (chloroform). For $C_{27}H_{38}O_{20}S$ (714·6) was calculated: 45·37% C, 5·36% H, 4·49% S; found: 45·57% C, 5·36% H, 4·40% S. ^{1}H NMR spectrum: 2·01 s (3 H), 2·05 s (3 H), 2·10 s (3 H), 2·12 s (6 H), 2·13 s (3 H), 2·14 s (3 H), 3·16 s (3 H), 4·90 dd ($J_{1,2}$ = 3·9 Hz, $J_{2,3}$ = 9·8 Hz, H-2), 5·06 t ($J_{3,4} = J_{4,5}$ = 9·8 Hz, H-4), 5·26 d ($J_{3',4'}$ = 7·8 Hz, H-3′), 5·52 dd ($J_{3',4'}$ = 7·8 Hz, $J_{4',5'}$ = 4·4 Hz, H-4′), 5·48 t ($J_{2,3} = J_{3,4}$ = 9·8 Hz, H-3), 5·68 d ($J_{1,2}$ = 3·9 Hz, H-1). ^{13}C NMR spectrum: 20·4 q (7 C), 38·5 q, 61·7 t, 62·2 t, 63·4 t, 68·1 d, 68·5 d, 69·4 d, 70·0 d, 73·3 d, 77·7 d, 78·5 d, 89·8 d, 102·5 s, 169·3 s (2 C), 169·6 s (2 C), 169·8 s (2 C), 170·6 s.

1′,2,3,3′,4,4′,6-Hepta-O-acetyl-6′-O-methanesulfonylsucrose (*VIII*)

Using the same procedure as for compounds *VI* and *VII*, we prepared from compound *I* (50 mg) the compound *VIII* (56 mg), $[\alpha]_D$ +50·0° (chloroform). For $C_{27}H_{38}O_{20}S$ (714·6) was calculated:

45·37% C, 5·36% H, 4·49% S; found: 45·22% C, 5·52% H, 4·35% S. ^{1}H NMR spectrum: 2·02 s (3 H), 2·05 s (3 H), 2·12 s (12 H), 2·18 s (3 H), 3·11 s (3 H), 4·87 dd ($J_{1,2}$ = 3·6 Hz, $J_{2,3}$ = 10·4Hz, H-2), 5·04 t ($J_{3,4} = J_{4,5}$ = 9·2 Hz, H-4), 5·18 dd ($J_{3',4'}$ = 6·1 Hz, $J_{4',5'}$ = 4·3 Hz, H-4'), 5·45 dd ($J_{2,3}$ = 10·4 Hz, $J_{3,4}$ = 9·2 Hz, H-3), 5·52 d ($J_{3',4'}$ = 6·1 Hz, H-3'), 5·63 d ($J_{1,2}$ = = 3·6 Hz, H-1). ^{13}C NMR spectrum: 20·7 q (7 C), 37·4 q, 62·0 t, 62·6 t, 68·2 d, 68·7 t, 68·8 d, 69·4 d, 70·2 d, 74·8 d, 75·4 d, 78·8 d, 90·3 d, 104·0 s, 169·6 s (2 C), 170·1 s (4 C), 170·8 s.

1',2,3,3',4,6,6'-Hepta-O-acetyl-4'-O-methanesulfonylsucrose (*IX*)

The same procedure described under compounds *VI* and *VII*, applied to compound *II* (123 mg), pyridine (2 ml), and methanesulfonyl chloride (0·15 ml), provided the compound *IX* (138 mg). After purification on a silica gel column (15 g) in the system benzene–ethanol 100 : 2, the optical rotation was $[\alpha]_D$ +48·0° (chloroform). For $C_{27}H_{38}O_{20}S$ (714·6) was calculated: 45·37% C, 5·36% H, 4·49% S; found: 45·27% C, 5·41% H, 4·39% S. ^{1}H NMR spectrum: 2·02 s(3 H), 2·05 s (3 H), 2·10 s (6 H), 2·12 s (6 H), 2·20 s (3 H), 3·12 s (3 H), 4·85 dd ($J_{1,2}$ = 3·1 Hz, $J_{2,3}$ = 10·4 Hz, H-2), 5·06–5·50 mt (4 H, H-3, H-4, H-3', H-4'), 5·67 d ($J_{1,2}$ = 3·1 Hz, H-1). ^{13}C NMR spectrum: 20·7 q (7 C), 38·7 q, 61·8 t, 62·7 t, 63·2 t, 68·3 d, 68·6 d, 69·6 d, 70·1 d, 74·9 d, 77·9 d, 78·9 d, 89·9 d, 103·3 s, 169·5 s (2 C), 170·0 s (3 C), 170·5 s, 170·7 s.

1',2,3,3',4',6,6'-Hepta-O-acetylsucrose (*III*)

1 ml of solution prepared by mixing of acetanhydride (1·2 ml) and pyridine (10 ml) was added to the mixture of compound *XIII* (574 mg, refs[5,13]) and pyridine (15 ml) at −70°C. The mixture was kept 72 h at −15°C, then decomposed by water, solvents removed, toluene added and evaporated again. The residue (640 mg) was chromatographed on a silica gel column (60 g, eluent chloroform). Octa-O-acetylsucrose (170 mg, 26%) and compound *III* (413 mg, 68%), $[\alpha]_D$ +44·9° (chloroform) were obtained. Literature[2] gives $[\alpha]_D$ +46·3° (chloroform). ^{1}H NMR data did not differ from those published[2]. Methanesulfonyl derivative *XIV* prepared from *III* by usual procedure had m.p. 85–88°C and $[\alpha]_D$ +48·1° (chloroform). Literature[14] gives m.p. 94–95°C, $[\alpha]_D$ +25·2° (chloroform), ref.[16] gives m.p. 91–93°C, $[\alpha]_D$ +51°C (chloroform).

2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl-1,6-di-O-
-acetyl-3,4-anhydro-β-D-lyxo-hexulofuranoside (*X*)

Solution of methanesulfonyl derivative *IX* (432 mg) in methanolic sodium methoxide (5 ml, 1 mol l^{-1}) was heated to boiling for 1 min. The solvent was evaporated and pyridine (12 ml) plus acetanhydride (4 ml) were added. The mixture was left standing for 12 h at room temperature, then poured onto ice and the product was taken up with chloroform. The residue after removing the solvent was purified using a silica gel (30 g) column chromatography in the system ether–light petroleum 4 : 1. The yield was 337 mg (95%) of the anhydro derivative *X*, $[\alpha]_D$ +57·0° (chloroform), according to NMR identical with the authentic sample[5,12].

Kinetic Studies of Deacetylation of Octa-O-acetylsucrose,
Compounds *I*, *II*, and the Mixture of Compounds *XI* and *XII*

The detector response factors were determined by measuring a standard solution containing weighted amounts of octa-O-acetylsucrose and compounds *I*, *II*, and *V*. They were set equal to one with the accuracy sufficient for further measurements. Capacity factors: methyl acetate 1·42, penta-O-acetylsucroses 1·47 and 1·63, *IV* 2·16, *V* 2·81, *XI* 3·53, *II* 4·00, *I* and *XII* 4·47, octa-O-

acetylsucrose 8·53. For kinetic runs, the solution of studied compound (400 mg) in methanol (10 ml) was shaken with impregnated aluminum oxide[3,5] (1·0 g) in a stopped test tube (conical ground glass joint). Every hour was the sample centrifugated (33·3 Hz, 1 min), 10 µl sample was taken and the solvent was evaporated. The residue was dissolved in 20 µl of 50% aqueous methanol and 2 µl aliquots were used for an assay. Last analysis was performed after 12 h of shaking and 9 h of standing at room temperature.

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REFERENCES

1. Ballard J. M., Hough L., Richardson A. C.: Carbohyd. Res. *24*, 152 (1972).
2. Ballard J. M., Hough L., Richardson A. C.: Carbohyd. Res. *34*, 184 (1974).
3. Čapek K., Čapková J., Kvapilová E., Vydra T., Bláhová M., Ranný M.: Czech 223 440.
4. Čapek K., Vydra T., Ranný M., Sedmera P.: This Journal *50*, 2191 (1985).
5. Čapek K., Vydra T., Čapková J., Ranný M., Bláhová M., Sedmera P.: This Journal *50*, 1039 (1985).
6. Otake T.: Bull. Chem. Soc. Jap. *43*, 3199 (1970).
7. Buchanan J. G., Cummerson D. A., Turner D. M.: Carbohyd. Res. *21*, 283 (1972).
8. Goodlet V. W.: Anal. Chem. *37*, 431 (1965).
9. Trehan I. R., Monder C., Bose A. K.: Tetrahedron Lett. *1968*, 67.
10. Bose A. K., Srivanasan P. R.: Tetrahedron *31*, 3025 (1975).
11. Khan R.: Adv. Carbohyd. Chem. Biochem. *33*, 235 (1976).
12. Khan R., Jenner M. R., Lindseth H.: Carbohyd. Res. *65*, 99 (1978).
13. Khan R., Mufti K. S., Jenner M. R.: Carbohyd. Res. *65*, 109 (1978).
14. Khan R.: Carbohyd. Res. *25*, 232 (1972).
15. Khan R.: Carbohyd. Res. *32*, 375 (1974).
16. Fairclough P. H., Hough L., Richardson A. C.: Carbohyd. Res. *40*, 285 (1975).

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