PARTIAL ESTERIFICATION OF METHYL 3-ACETAMIDO-3,6-DIDEOXY-β-D-GALACTOPYRANOSIDE AND METHYL 3-ACETAMIDO-3,6-DIDEOXY-β-D-TALOPYRANOSIDE*

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On partial acetylation of methyl 3-acetamido-3,6-dideoxy-β-D-galactopyranoside (*I*) a mixture of 2,4-di-O-acetyl (*X*), 2-O-acetyl (*XI*) and 4-O-acetyl derivative (*XII*) is formed. The ratios of the rate constants of this reaction system have been calculated. While in the acetylation with acetic anhydride in pyridine the relative reactivity of hydroxyl groups does not practically change in the course of the two-stage reaction, in the acetylation with acetyl chloride in pyridine the ratio of the reactivities of the hydroxyl groups in the starting substance *I* is opposite to that of corresponding mono-O-acetyl derivatives *XI* and *XII*. Consequently, in the latter case more 4-O-acetyl derivative *XII* is obtained when the conversion degree is low, while at higher conversions 2-O-acetyl derivative *XI* predominates. Partial mesylation of substance *I* affords in addition to di-O-methanesulfonyl derivative *XVI* almost exclusively 4-O-methanesulfonyl derivative *XV*. Methyl 3-acetamido-3,6-dideoxy-β-D-talopyranoside (*II*) and acetic anhydride in pyridine gives in addition to 2,4-di-O-acetyl derivative *IV* also 2-O-acetyl *XVII* and 4-O-acetyl derivative *XVIII* in a 5·7 : 1 ratio; with acetyl chloride, *XVII* is formed almost exclusively. Partial deacetylation of substance *IV* on alkaline alumina gives 4-O-acetyl derivative *XVIII* exclusively, while in the case of substance *X* the ratio *XII* : *XI* is 5·4 : 1. The position of acetyl or methanesulfonyl groups in single partially esterified derivatives was proved by ^{1}H-NMR spectra and chemical reactions. In the reaction of methyl 3-acetamido-3,6-dideoxy-2-O-methanesulfonyl-β-D-galactopyranoside (*VI*) with sodium acetate methyl 2-acetamido-2,6-dideoxy-β-D-idopyranoside (*IX*) is obtained in addition to acetamidotaloside *II*.

In connection with the study of partial acetylation[1-7] of sugar compounds we investigated also the esterification of methyl 3-acetamido-3,6-dideoxy-β-D-glucopyranoside and methyl 3-acetamido-3,6-dideoxy-β-D-mannopyranoside and came to the conclusion[7] that the acetamido group in the position 3 is unable to isolate sufficiently the two hydroxyl groups in the positions 2 and 4, so that the reactivity of the respective hydroxyl group in these derivatives changes in the course of the multistage reaction. Consequently, the experimentally determined ratio of amounts of mono-O-acetyl derivatives is not consistent with the ratio of reactivities of the hydroxyl groups in the starting substance acetylated. In such case, a complete kinetic

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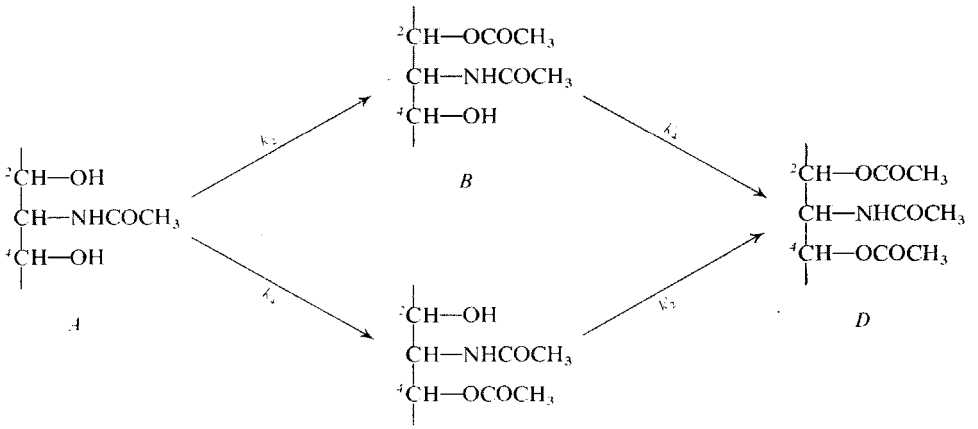
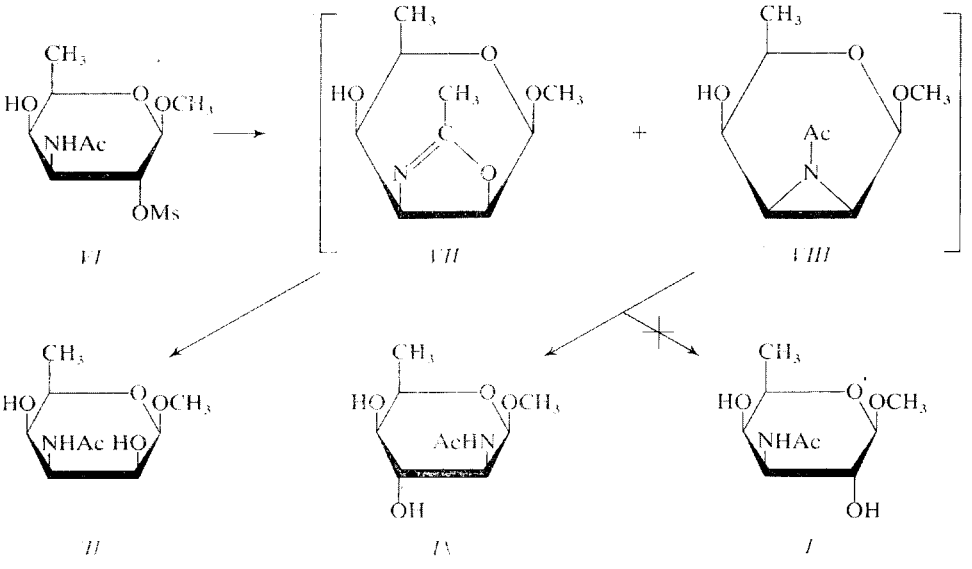
solution of the given reaction system (Scheme 1) is necessary for the understanding of the effects influencing the reactivity of the hydroxyl groups of the diol; the procedure required for this, based on the exploitation of relatively little experimental data, is described in our preceding paper[8]. In the present study we make use of this procedure for the esterification of methyl 3-acetamido-3,6-dideoxy-β-D-galactopyranoside (*I*) and methyl 3-acetamido-3,6-dideoxy-β-D-talopyranoside (*II*).



SCHEME 1

Acetamido derivative *I* was prepared by nitromethane condensation of the dialdehyde formed by periodate cleavage of methyl 6-deoxy-β-D-glucopyranoside[9]. Acetamido derivative *II* which is obtained from the condensation mentioned in a very low yield was prepared on reaction of methyl 3-acetamido-3,6-dideoxy-2,4-di-O-methanesulfonyl-β-D-glucopyranoside (*III*) with sodium acetate in aqueous 2-methoxyethanol[9]. When this reaction is carried out on a larger scale and the reaction product is acetylated with acetic anhydride in pyridine we isolated now – in addition to the main product, methyl 3-acetamido-2,4-di-O-acetyl-3,6-dideoxy-β-D-talopyranoside (*IV*) and traces of two unidentified substances – also another N-acetyl-per-O-acetyl derivative *V* in 5% yield, for which the structure of 2-acetamido-2-deoxy derivative follows unambiguously from the ^{1}H-NMR spectra (the band $H_{(2)}$ is split to an octet with coupling constants $J_{1,2} = 2{\cdot}5$, $J_{2,3} = 5{\cdot}1$, $J_{2,NH} = 9{\cdot}0$ Hz). The magnitude of the constants $J_{2,3} = J_{3,4} = 5{\cdot}1$ Hz which exludes the *cis*-arrangement of the hydrogen atoms $H_{(2)}$ and $H_{(3)}$, or $H_{(3)}$ and $H_{(4)}$ (see[10]) is decisive for the assignment of β-D-*ido* configuration; it also indicates that substance *V* is not conformationally pure in chloroform solution. The formation of 2-acetamidoidoside parallelly with 3-acetamidotaloside can be explained by the fact that during the solvolysis of the methanesulfonyloxy group in the position 2 of the temporarily formed[9] methyl 3-aceta-

mido-3,6-dideoxy-2-O-methanesulfonyl-β-D-galactopyranoside (*VI*) the vicinal *trans*--acetamido group participates not only by its carbonyl group, but also by its nitrogen atom, probably because the participation of the carbonyl oxygen of the acetamido group in the nucleophilic substitution on the carbon atom $C_{(2)}$ (which leads to a derivative with β-D-*talo* configuration) is made difficult by the sterical and the polar effect of the anomeric centre. Thus, in addition to oxazoline derivative with β-D-*talo* configuration, *VII*, N-acetylepimine *VIII* is probably also formed (Scheme 2), which hydrolyses under the reaction conditions to methyl 2-acetamido-2,6-dideoxy-β-D--idopyranoside (*IX*). In the mixture after acetylation we were unable to detect the second possible product of the opening of the epimino derivative *VIII*, *i.e.* methyl 3-acetamido-2,4-di-O-acetyl-3,6-dideoxy-β-D-galactopyranoside (*X*), which proves the exclusive diaxial opening of the aziridine ring of substance *VIII*. The participation of the acetamido group leading to the N-acetylated epimino derivatives is common, for example in the reactions of acetamido compounds, containing a vicinal *trans*--sulfonyloxy group or halogen atom, with sodium methoxide in methanol (*cf.*[11]); however, it has not yet been described when the reaction is carried out under the conditions of solvolysis in aqueous 2-methoxyethanol. Neither of the intermediates *VII* or *VIII* could be detected in the reaction mixture; the preparation of N-acetylepimine *VIII* in a reasonable yield, performed on reaction of compound *VI* with sodium methoxide is impossible due to the rearrangement described[12] for the α-anomer of compound *VI*.



VI *VII* *VIII*

II *IV* *I*

SCHEME 2

Partial acetylation of acetamidogalactoside *I* both with acetyl chloride and with acetic anhydride in pyridine gave a mixture of starting compound *I*, methyl 3-acetamido-2-O-acetyl-3,6-dideoxy-β-D-galactopyranoside (*XI*), methyl 3-acetamido-4-O-acetyl-3,6-dideoxy-β-D-galactopyranoside (*XII*), and 2,4-di-O-acetyl derivative *X*. These mixtures were separated on a silica gel column and the results of the chromatography are presented in Table I. The position of O-acetyl group in derivatives *XI* and *XII* was determined from the results of their reaction with methanesulfonyl chloride in pyridine; methyl 3-acetamido-4-O-acetyl-3,6-dideoxy-2-O-methanesulfonyl-β-D-galactopyranoside (*XIII*) obtained from derivative *XII* was identical with the product of acetylation of compound *VI* prepared in our preceding paper[9]. By the same reaction, 2-O-acetyl derivative *XI* afforded methyl 3-acetamido-2-O-acetyl-3,6-dideoxy-4-O-methanesulfonyl-β-D-galactopyranoside (*XIV*) the catalytic deacetylation of which led to methyl 3-acetamido-3,6-dideoxy-4-O-methanesulfonyl-β-D-galactopyranoside (*XV*). Substance *XV* was also obtained as the dominant product of partial mesylation of acetamidogalactoside *I* in addition to 2-O-methanesulfonyl derivative *VI* and methyl 3-acetamido-3,6-dideoxy-2,4-di-O-methanesulfonyl-β-D-galactopyranoside (*XVI*) (Table I).

TABLE I

The Composition of the Reaction Mixture after Partial Esterification in Pyridine

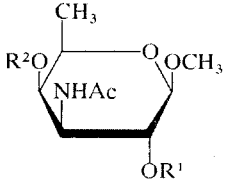
Each experiment was carried out to a similar degree of conversion at least twice; in the table only a single series of the data obtained is presented.

Reagent[a]	Regenerated %	2-OR %	4-OR %	2,4-Di-OR %	2-OR/4-OR	DS[b]	Total %
Methyl 3-acetamido-3,6-dideoxy-β-D-galactopyranoside (*I*)							
Ac_2O	30·3	33·3	11·1	25·6	3·00	0·96	100
AcCl	22·0	14·9	11·9	51·2	1·13	1·29	100
AcCl	79·8	8·0	9·2	2·8	0·87	0·24	99·8
MsCl	1·0	3·1	75·4	20·3	0·04	1·19	99·8
MsCl	51·0	3·6	41·9	3·4	0·09	0·52	99·9
Methyl 3-acetamido-3,6-dideoxy-β-D-talopyranoside (*II*)							
Ac_2O	53·4	35·2[c]	6·2[c]	3·3	5·69[c]	0·48	98·1
AcCl	28·8	63·7[c]	0·4[c]	5·0	150[c]	0·74	97·9

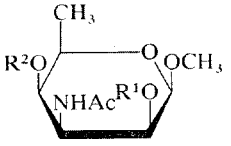
[a] Ac_2O acetic anhydride, AcCl acetyl chloride, MsCl methanesulfonyl chloride; [b] degree of substitution; [c] the ratio of mono-O-acetyl derivatives was determined from the ^{1}H-NMR spectra in deuteriochloroform by integration of the corresponding bands, at 100 Hz sweep width.

The structure of derivative *XIV* was confirmed on the basis of the comparison of ^{1}H-NMR spectrum of this substrance with the published spectra[9] of 2-O-methanesulfonyl-4-O-acetyl-, or 2,4-di-O-acetyl derivative (*XIII* or *X*, resp.). The upfield shift of the $H_{(4)}$ proton signal by 0·44 or 0·31 p.p.m., together with the downfield shift of the doublet signal of the methyl group on carbon atom $C_{(5)}$ by 0·12 p.p.m. corresponds[7] completely to the proposed structure. From the coupling constant value $J_{2,3} = 11{\cdot}0$ Hz, measured in the ^{1}H-NMR spectrum of compound *XIV* it follows clearly that this derivative exists in solutions in conformation $^4C_1$ exclusively.

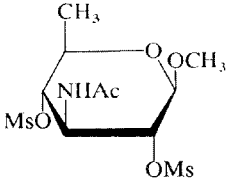
Acetylation of methyl 3-acetamido-3,6-dideoxy-β-D-talopyranoside (*II*) with acetic anhydride in pyridine gave in addition to the unreacted starting compound and 2,4-di-O-acetyl derivative *IV* also a mixture of methyl 3-acetamido-2-O-acetyl-3,6-dideoxy-β-D-talopyranoside (*XVII*) and methyl 3-acetamido-4-O-acetyl-3,6-dideoxy-β-D-talopyranoside (*XVIII*), which could not be separated chromatographically. Their mutual ratio in the fraction of mono-O-acetyl derivatives was determined by ^{1}H-NMR spectroscopy from the ratio of the areas of $H_{(1)}$ hydrogen atoms bands, from the doublets of methyl groups on carbon atom $C_{(5)}$, and from the ratio of the



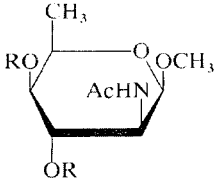
I, $R^1 = R^2 = H$
VI, R^1 = Ms; $R^2 = H$
X, $R^1 = R^2$ = Ac
XI, R^1 = Ac; $R^2 = H$
XII, $R^1 = H$; R^2 = Ac
XIII, R^1 = Ms; R^2 = Ac
XIV, R^1 = Ac; R^2 = Ms
XV, $R^1 = H$; R^2 = Ms
XVI, $R^1 = R^2$ = Ms



II, $R^1 = R^2 = H$
IV, $R^1 = R^2$ = Ac
XVII, R^1 = Ac; $R^2 = H$
XVIII, $R^1 = H$; R^2 = Ac



III



V, R = Ac
IX, R = H

areas of the signals $H_{(2)}$ (*XVII*)/$H_{(4)}$ (*XVIII*). The results are given in Table I. The structures of mono-O-acetyl derivatives *XVII* and *XVIII* were assigned on the basis of the known difference of the chemical shift of protons on the carbon atom carrying the hydroxyl group or the acetoxyl group, respectively; in our case it was a downfield shift of about 1·7 p.p.m. To obtain the authentic spectra of pure compounds *XVII* and *XVIII* the fact has been made use of that on acetylation of acetamidotaloside *II* with acetyl chloride in pyridine 2-O-acetyl derivative *XVII* is formed almost exclusively (Table I), while from partial deacetylation of di-O-acetyl derivative *IV* on alkaline alumina in benzene practically pure 4-O-acetyl derivative *XVIII* is obtained in relatively high yield (33·3%), in addition to the unreacted starting di-O-ace--acetyl derivative *IV* (53·6%); the ratio of *XVIII* : *XVII* was determined from the ^{1}H-NMR spectra as 17·5 : 1. The coupling constant values, determined for compounds *XVII* and *XVIII* do not permit – with respect to the *cis*-arrangement of all hydrogen atoms of the pyranoside ring – an expression of any conclusions regarding the conformation of these compounds. The chemical shift of the acetoxy group protons[13] (δ 2·17 in both cases) indicates that it is the chair conformation $^4C_1$ which would predominate in conformational equilibria. The acetamido group resonance is evidently affected[14] by the presence of the vicinal hydroxyl group, so that the corresponding band (δ 1·99) appears in the region assigned in N-acetyl--per-O-acetyl derivatives to the group in axial position.

It is known that the deacetylation on alkaline alumina[1-5] takes place in the case of methyl 3-acetamido-2,4-di-O-acetyl-3,6-dideoxy-α-D(L)-hexopyranosides almost exclusively under formation of 4-O-acetyl derivatives. This rule, the validity of which was also demonstrated for the derivatives with β-D-*gluco* and β-D-*manno* configuration[7], also applies – as shown above – for methyl 3-acetamido-2,4-di-O-acetyl--3,6-dideoxy-β-D-talopyranoside (*IV*). A similar result was obtained from the analogous reaction of 2,4-di-O-acetyl derivative *X*, where 20·9% of 4-O-acetyl derivative *XII* and only 3·9% of 2-O-acetyl derivative *XI* have been isolated, in addition to 63·3% of unreacted starting substance *X*.

As it is evident from Table I, a considerable amount of 2,4-di-O-acetyl- or 2,4-di-O--methanesulfonyl derivative (*X*) or (*XVI*) is formed during the described esterifications of methyl 3-acetamido-3,6-dideoxy-β-D-galactopyranoside (*I*) with approximately one equivalent of the reagent. As the disubstituted substances necessarily originate from partially esterified derivatives *B* and *C* (Scheme 1), the mentioned distribution of the products (Table I) need not be decisive for the reactivity ratios of hydroxyl groups in substance *I*. Therefore, we have carried out esterifications to the second stage, *i.e.* reactions of mixtures of mono-O-acetyl or mono-O-methanesulfonyl derivatives of known composition with less than one equivalent of reagent, and we calculated by a described procedure[7,8] all ratios of rate constants describing the system of the reactions in Scheme 1. For acetylation with acetic anhydride in pyridine the following values of the rate constants ratios have been calculated: $k_2/k_4 = 2·1$,

$k_2'/k_4' = 2{\cdot}2$, $k_2'/k_2 = 1{\cdot}6$, $k_2'/k_4 = 3{\cdot}4$, $k_4'/k_2 = 0{\cdot}7$, $k_4'/k_4 = 2{\cdot}1$. For the reaction with acetyl chloride in pyridine the values were $k_2/k_4 = 0{\cdot}8$, $k_2'/k_4' = 1{\cdot}6$, $k_2'/k_2 = 3{\cdot}6$, $k_2'/k_4 = 2{\cdot}9$, $k_4'/k_2 = 2{\cdot}2$, $k_4'/k_4 = 1{\cdot}9$. In the reaction of acetamido derivative *I* with methanesulfonyl chloride in pyridine the reactivity of the hydroxyl groups differs considerably; therefore we chose the procedure of calculations[8] from several mesylations, carried out to different degrees of substitution DS. Two of them are given in Table I. This procedure gave the following values for the rate constant ratios: $k_2/k_4 = 0{\cdot}1$, $k_2'/k_4' = 0{\cdot}15$, $k_2'/k_2 = 0{\cdot}07$, $k_2'/k_4 = 0{\cdot}07$, $k_4'/k_2 = 4$, $k_4'/k_4 = 0{\cdot}4$.

In esterifications of acetamidotaloside *II* the very sight of the values given in Table I indicates considerable differences in the reactivities of the hydroxyl groups. In other words, the data indicate high values of some rate constant ratios. However, for required accurate values of all rate constant ratios to be calculated[8], a large set of acetylation experiments with various degrees of substitution would be necessary, due to the complicated experimental evaluation and the accuracy of the NMR determination of the ratios of mono-O-acetyl derivatives *XVII* and *XVIII*. Unfortunately, the accessibility of the starting diol was too bad to make a performance of these experiments possible.

From the values of the rate constant ratios of the acetylation of acetamidogalactoside *I* with acetic anhydride in pyridine it is evident that the reactivity of a certain hydroxyl group increases when the other hydroxyl (in γ-position) is substituted by an acetoxy group ($k_2'/k_2 = 1{\cdot}6$, $k_4'/k_4 = 1{\cdot}5$). The increase in reactivity in the position 2 and in the position 4 is practically equal, which is reflected, of course, in the equal ratio of reactivities of the hydroxyl groups when esterified to the first and the second stage ($k_2/k_4 \approx k_2'/k_4'$). In contrast to this in the acetylation with acetyl chloride in pyridine the ratios of the reactivities to the first and the second stage not only differ, but they also have the "opposite sign" ($k_2 < k_4$ and $k_2' > k_4'$). This means that in this case the mixture should contain at low conversion degrees (in contrast to the described distribution with DS = 1·29, see Table I) more 4-O-acetyl derivative *XII* ($k_2/k_4 = 0{\cdot}8$), which disappears, however, substantially faster ($k_2'/k_2 = 3{\cdot}6$) than 2-O-acetyl derivative *XI* ($k_4'/k_4 = 1{\cdot}9$). Indeed, with 0·25 equivalents of acetyl chloride we obtained slightly more 4-O-acetyl derivative *XII* than 2-O-acetyl derivative *XI* (Table I). Then, it is possible to calculate the degree of substitution DS at which an equal amount of both mono-O-acetyl derivatives would be obtained. Theoretically[8], 15·5% each, together with 50·1% of the starting compound *I* should be isolated at DS = 0·67. Of course, the curve of the dependence of the concentration of substance *XI* or *XII* on the concentration of substance *I* is so flat in this region that from the practical point of view a negligible difference in the concentrations of derivatives *XI* and *XII* may be obtained (also with respect to the accuracy of the measurement) within a rather broad range of acetyl chloride consumption. From the mentioned course of the acetylation of acetamidogalactoside *I* with acetyl chloride in pyridine

it is evident that the use of experimentally obtained ratio of the amounts of mono-O-acetyl derivatives at only one conversion degree as a criterion of the reactivity of the hydroxyl groups in the starting diol could lead to completely wrong conclusions.

The fact observed that the reactivity of the hydroxyl group in methyl 3-acetamido-3,6-dideoxyhexopyranosides changes in the course of two-stage reaction, *i.e.* that the acetamido group is not able of separating two hydroxyl groups incapable of a direct interaction, is also evident from the comparison of the results of acetylation of substance *I* with acetic anhydride in pyridine with the described result[15] of acetylation of methyl 3,6-dideoxy-β-D-*xylo*-hexopyranoside. Should we suppose that in abequoside the relationship $k_2 \approx k_2'$ and $k_4 \approx k_4'$ already applies, then a large excess of 2-O-acetyl derivative (38·5%) over 4-O-acetyl derivative (2·4%) in this compound (even after accounting for the simultaneous isolation of 9·6% of 2,4-di-O-acetyl derivative[15]) indicates that the presence of the acetamido group on the carbon atom $C_{(3)}$ in substance *I* will either considerably slow down the reactivity of the *trans* $O_{(2)}H$ group, or – on the contrary – enhance the reactivity of the *cis* $O_{(4)}H$ group.

In the reaction of acetamidogalactoside *I* with methanesulfonyl chloride in pyridine a high percentage of the 4-O-methanesulfonyl derivative *XV* obtained is caused by the higher reactivity of $O_{(4)}H$ both in the first step ($k_2/k_4 = 0{\cdot}1$) and in the second step ($k_2'/k_4' = 0{\cdot}15$). In both positions a mild decrease in reactivity is observed after mesylation in the γ-position.

No doubt the above facts cannot be obtained directly from the experimentally observed values, *i.e.* from the data given in Table I. On the other hand, in the case of the derivative *II* with β-D-*talo* configuration the situation is slightly different. The small amount of the disubstituted derivative *IV* in the acetylation with acetyl chloride in pyridine permits us to state unambiguously that k_2 will be higher then k_4 ($k_2/k_4 > 1$) or than k_4', respectively. However, the ratios of other rate constants cannot be deduced, even in this simplified case, from the total material balance. The preferential acetylation of the $O_{(2)}H$ group in methyl 3-acetamido-3,6-dideoxy-β-D-talopyranoside (*II*) indicates a considerable effect of the orientation of the anomeric methoxyl group on the reactivity of the hydroxyl groups with this reagent, because the α-L-*ido* isomer, reacting in the conformation with the equal arrangement of hydroxyl groups (the mere change in configuration of the acetamido group on the symmetrically substituted carbon atom $C_{(3)}$ should not affect the reactivity ratio of both hydroxyl groups too much), afforded[3] – at only slightly higher conversion degree – 44% of di-O-acetyl derivative together with mono-O-acetyl derivatives in a 1·54 : 1 ratio, in favour of 2-O-acetyl derivative (14·6% or 9·5%, resp.). For the reaction of acetamidotaloside *II* with acetic anhydride in pyridine it may be stated on the basis of experimental data that the value of k_2/k_4 ratio is approximately equal to 5, because the amount of di-O-acetyl derivative *IV* is negligible

(Table I). Hence, the reactivity of $O_{(2)}H$ is substantially lower in this case than in the reaction with acetyl chloride in pyridine.

The ratios mentioned of rate constants have in addition to their theoretical importance also a considerable preparative relevancy. The changes in the rate constants of the esterification of particular hydroxyl groups during the multistage reaction result in the maximum possible yield of the partially esterified product not always having to correspond to the degree of substitution DS = 1. In other words, the reaction with the commonly used 1–1·2 equivalents of the reagent need not by any means be preparatively most advantageous for the preparation of a certain mono-O-acyl derivative. The maximum possible yield of monosubstituted derivative (together with the corresponding consumption of the reagent), *i.e.* virtually the extreme of the dependence of the concentration of substance *B*, or *C*, on the concentration of the starting substance *A* (Scheme 1) can be calculated[8], however, from the mentioned rate constants ratios, which describe quite accurately the reaction course from DS = 0 to DS = 2. Under the assumption of the validity of the values determined by us we calculated that on the acetylation of derivative *I* with acetyl chloride in pyridine maximally 15·6% of 4-O-acetyl derivative *XII* can be prepared (together with 16·0% of *XI* and 22·5% of *X*) at DS = 0·77, *i.e.* – under the assumption that no side reaction took place with acetyl chloride in pyridine – in the reaction with 0·8 equivalents of the acylating agent only (but not with the usual 1,2-equivalents). We carried out this experiment, and the actual yield of substance *XII* practically did not differ from the theoretical one. However, it should be noted that in this case the decrease of the yield in dependence on additional amounts of acetyl chloride is not too conspicuous (a flat maximum). By the same procedure the yield of *XI* (max) 16·5% may also be computed (together with 15·2% of *XI* and 31·4% of *X*, DS = 0·94) and for the acetylation with acetic anhydride *XII* (max) 11·3% (together with 31·8% of *XI* and 18·3% of *X*, DS = 0·80), or *XI* (max) 33·7% (together with 10·5% of *XII* and 30·7% of *X*, DS = 1·06). Hence, from the preparative point of view acetylation with acetic anhydride is more suitable for the preparation of 2-O-acetyl derivative *XI*, while acetyl chloride in pyridine is more convenient for the preparation of 4-O-acetyl derivative *XII*. From the mesylation of derivative *I* maximum 76·1% of 4-O-methanesulfonyl derivative *XV* would be obtained (together with 4·4% of *VI* and 14·9% of *XVI*, DS = 1·10) and not more than 5·6% of 2-O-methanesulfonyl derivative *VI* (together with 67·8% of *XV* and 6·1% of *XVI*, DS = 0·86). The data given in Table I for mesylation with 1·18 equivalents of methanesulfonyl chloride agree quite well with this calculation.

EXPERIMENTAL

The melting points were measured on a Kofler block and they are not corrected. Optical rotation was determined with an instrument of the Opton firm, at 20°C and at 0·5—1·0 g/100 ml concentrations. ^{1}H-NMR spectra were measured on a Varian XL-100-15 instrument in deuteriochloroform,

using tetramethylsilane as internal standard. Chemical shifts are given in δ-values, coupling constants in Hz. Chromatographies were carried out on silica gel of Lachema (Brno) 70—200 μm; thin-layer chromatography was carried out with silica gel according to Stahl (Merck, Darmstadt), 10—40 μm, using plates of 27 × 75 mm dimension and a layer 0·2—0·3 mm thick. Substances were detected by spraying with 1% cerium(IV) sulfate in 10% sulfuric acid and subsequent mineralization. The solvents were evaporated on a rotatory evaporator in a water-pump vacuum, at a temperature not exceeding 50°C. The light petroleum used for crystallization had b.p. 45—60 °C Samples for analysis were dried at 20—50°C and 0·05—0·1 Torr. The calculations of rate constants ratios were performed on a Hewlett-Packard 2116B computer.

Acetylation of Acetamidogalactoside *I*

a) *With acetic anhydride in pyridine*: Acetic anhydride (0·20 ml; 2·12 mmol) was added at −70°C to a mixture of 400 mg (1·82 mmol) of acetamidogalactoside *I* (ref.[9]) and 10 ml of pyridine, and the mixture was allowed to stand at −16°C for 48 hours and then at 0°C for 24 hours, and eventually at room temperature for another 24 hours. After decomposition with water the mixture was evaporated several times with water and finally with toluene and the residue was dried in a vacuum (oil pump) and introduced into a column containing 30 g of silica gel. After washing of the column with 100 ml of benzene, elution was started with benzene–ethanol mixture 100 : 5, which eluted 142 mg (0·47 mmol, 25·6%) of di-O-acetyl derivative *X* (ref.[9]), 158·5 mg (0·60 mmol, 33·3%) of 2-O-acetyl derivative *XI*, 53 mg (0·20 mmol, 11·1%) of 4-O-acetyl derivative *XII*, and 120 mg (0·55 mmol, 30·0%) of the starting compound *I*. 2-O-Acetyl derivative *XI* was sublimated at 150°C/0·1 Torr, m.p. 167—168°C, $[\alpha]_D$ −21° (chloroform). For $C_{11}H_{19}NO_6$ (261·3) calculated: 50·57% C, 7·33% H, 5·36% N; found: 50·68% C, 7·42% H, 5·20% N. 4-O-Acetyl derivative *XII* was crystallized from ethyl acetate, m.p. 238—239°C (under sublimation), $[\alpha]_D$ +49° (chloroform). For $C_{11}H_{19}NO_6$ (261·3) calculated: 50·57% C, 7·33% H, 5·36% N; found: 50·77% C, 7·50% H, 5·46% N.

b) *With acetyl chloride in pyridine*: Acetyl chloride (170 μl; 2·40 mmol) was added at −70°C to a mixture of 400 mg (1·82 mmol) of substance *I* (ref.[9]) in 10 ml of pyridine and the mixture was allowed to stand at −16°C for 48 hours. then at 0°C for 8 hours. After decomposition with water it was evaporated repeatedly with water and eventually with toluene. The residue was applied on the silica gel column (30 g) and chromatographed in the same manner as above. Yield, 284 mg (0·94 mmol, 51·2%) of di-O-acetyl derivative *X*, 71 mg (0·27 mmol, 14·9%) of 2-O-acetyl derivative *XI*, 57 mg (0·22 mmol, 11·9%) of 4-O-acetyl derivative *XII*, and 88 mg (0·40 mmol, 22·0%) of the starting substance *I*.

Acetylation of a Mixture of 2-O-Acetyl Derivative *XI* and 4-O-Acetyl Derivative *XII*

a) *With acetic anhydride in pyridine*: A mixture of 20·07 mg of 2-O-acetyl derivative *XI* and 19·30 mg of 4-O-acetyl derivative *XII* (0·151 mmol of mixture) was dissolved in 1 ml of pyridine, cooled at −70°C, and additioned with 8 μl of acetic anhydride. After 48 hours at −16°C, 24 hours at 0°C, and 24 hours at room temperature, the mixture was worked up in the same manner as described in the acetylation of substance *I* with acetic anhydride in pyridine. After chromatographic separation on a column of silica gel (7 g) 12·47 mg of compound *X*, 16·13 mg of 2-O-acetyl derivative *XI*, and 11·97 mg of 4-O-acetyl derivative *XII* were obtained; 80·4% of substance *XI* and 62·0% of substance *XII* remained unreacted.

b) *With acetyl chloride in pyridine*: A mixture of 40·60 mg of 2-O-acetyl derivative *XI* and 22·96 mg of derivative *XII* (0·243 mmol of mixture) was dissolved in 1·5 ml of pyridine, then

cooled at −70°C, additioned with 9·8 μl of acetyl chloride (0·140 mmol), and allowed to stand identically as in the acetylation of substance *I* with acetyl chloride in pyridine (see above). After working up and chromatography on a column of 10 g of silica gel 23·70 mg of compound *X*, 29·00 mg of 2-O-acetyl derivative *XI*, and 13·48 mg of 4-O-acetyl derivative *XII* were obtained; 71·4% of 2-O-acetyl derivative *XI* and 58·6% of 4-O-acetyl derivative *XII* remained unreacted.

Mesylation of Acetamidogalactoside *I*

Methanesulfonyl chloride (0·1 ml; 1·27 mmol) was added to a mixture of 210 mg (0·96 mmol) of compound *I* (ref.[9]) in 10 ml of pyridine at −70°C and the mixture was allowed to stand at −16°C for 48 hours, and at 0°C for 24 hours. After decomposition with water it was evaporated repeatedly with water and finally with toluene. The residue was chromatographed on a silica gel column (20 g). After washing with 200 ml of benzene the column was eluted with benzene–ethanol 100 : 5. The following fractions were obtained: 73 mg (0·195 mmol, 20·3%) of 2,4-di-O-methanesulfonyl derivative *XVI*, 9·0 mg (0·034 mmol, 3·1%) of 2-O-methanesulfonyl derivative *VI* (ref.[9]), 215 mg (0·724 mmol, 75·4%) of 4-O-methanesulfonyl derivative *XV* and 2 mg (0·01 mmol, 1%) of the starting compound *I*. The mesylation of 145·1 mg of substance *I* was carried out in the same manner (using 40 μl of methanesulfonyl chloride). The results of the chromatography are given in Table I. 2,4-Di-O-methanesulfonyl derivative *XVI* was crystallized from acetone–light petroleum, m.p. 192—194°C (under decomposition), $[\alpha]_D$ +8° (chloroform). For $C_{11}H_{21}NO_9S_2$ (375·4) calculated: 35·20% C, 5·14% H; found: 35·20% C, 5·57% H. 4-O-Methanesulfonyl derivative *XV* was crystallized from ethanol, m.p. 188—189°C (under decomposition), $[\alpha]_D$ +72° (methanol). For $C_{10}H_{19}NO_7S$ (297·3) calculated: 40·40% C, 6·44% H, 4·71% N, 10·28% S; found: 40·30% C, 6·66% H, 4·71% N, 10·31% S.

Acetylation of Acetamidotaloside *II*

a) *With acetic anhydride in pyridine*: 0·18 ml (1·19 mmol) of acetic anhydride was added at −70°C to a mixture of 348 mg (1·59 mmol) of acetamidotaloside *II* (ref.[9]) and 10 ml of pyridine and the mixture was allowed to stand at −16°C for 48 hours, then at 0°C for 24 hours, and at room temperature for another 24 hours. The mixture was decomposed with water, evaporated several times with water, and finally with toluene. The residue was chromatographed on a column of silica gel (30 g) with benzene–ethanol 100 : 5. Gradually the following fractions were obtained: 16 mg (0·05 mmol, 3·3%) of di-O-acetyl derivative *IV* (ref.[9]), 172 mg (0·66 mmol, 41·4%) of a mixture of mono-O-acetyl derivatives *XVII* and *XVIII*, and 186 mg (0·85 mmol, 53·4%) of the starting compound *II*. We were unable to separate the mono-O-acetyl derivatives chromatographically; from the ^{1}H-NMR spectra, *i.e.* by integration of respective bands, the ratio *XVII* : *XVIII* was found to be equal 5·7 : 1, which corresponds to 35·2% of 2-O-acetyl derivative *XVII* and 6·2% of 4-O-acetyl derivative *XVIII* in the mixture after acetylation.

b) *With acetyl chloride in pyridine*: A solution of 348 mg (1·59 mmol) of compound *II* (ref.[9]) in 10 ml of pyridine was cooled to —70°C, 0·12 ml (1·69 mmol) of acetyl chloride was added to it and the mixture allowed to stand at —16°C for 48 hours, then at 0°C for 24 hours, and eventually at 20°C for another 5 hours. After addition of water the mixture was evaporated several times with water and finally with toluene and the residue was chromatographed as in the case *a*). From silica gel 24 mg (0·08 mmol, 5·0%) of compound *IV*, 266 mg (1·02 mmol, 64·1%) of a mixture of mono-O-acetyl derivatives *XVII* and *XVIII*, and 100 mg (0·456 mmol, 28·8%) of the starting compound *II* were eluted. From the ^{1}H—NMR spectrum of the fraction of mono-O-acetyl derivatives the ratio *XVII* : *XVIII* ≈ 150 : 1 was determined, corresponding to 63·7% of 2-O-acetyl derivative *XVII* and 0·4% of 4-O-acetyl derivative *XVIII* in the original mixture

after acetylation. Practically pure 2-O-acetyl derivative *XVII*, obtained in the form of a syrup, has $[\alpha]_D$ −92° (chloroform). For $C_{11}H_{19}NO_6$ (261·3) calculated: 50·57% C, 7·33% H, 5·36% N; found: 50·43% C, 7·12% H, 5·30% N. ^{1}H—NMR data: 1·37 (3 H, d, $J_{5,6}$ = 6·3, CH_3CH), 1·99 (3 H, s, CH_3CONH), 2·17 (3 H, s, CH_3COO), 3·52 (3 H, s, CH_3O), 6·60 (1 H, d, $J_{3,NH}$ = = 8·3, NH), 5·36 (1 H, q, $J_{1,2}$ = 1·1, $J_{2,3}$ = 3·2, $H_{(2)}$), 4·49 (1 H, d, $J_{1,2}$ = 1·1, $H_{(1)}$), 4·24 (1H, m, $J_{3,NH}$ = 8·3, $J_{2,3}$ = 3·2, $J_{3,4}$ = 3·2, $H_{(3)}$), 3·71 (1 H, o, $J_{4,5}$ = 1·0, $J_{5,6}$ = 6·3, $H_{(5)}$), ~3·44 (1 H, q, $H_{(4)}$).

Deacetylation of Di-O-acetyl Derivative *IV*

A mixture of 200 mg (0·66 mmol) of 2,4-di-O-acetyl derivative *IV*, 5·6 g of alkaline alumina, and 11 ml of benzene was allowed to stand at room temperature with occasional shaking for 7 days. After filtration off of alumina and its washing with benzene–ethanol 5 : 1 the combined filtrates were evaporated and chromatographed on a silica gel column (16 g) with benzene–ethanol 100 : 5 which eluted first 107 mg (0·35 mmol, 53·6%) of the starting compound *IV*, then 58 mg (0·22 mmol, 33·6%) of a mixture of mono-O-acetyl derivatives *XVII* and *XVIII*, and 20 mg (0·09 mmol, 13·9%) of acetamidotaloside *II*. From the ^{1}H-NMR spectrum of the fraction of mono-O-acetyl derivatives it was found that it is a mixture of *XVIII* and *XVII* in approximately 17·4 : 1 ratio, corresponding to 31·8% of compound *XVIII* and 1·8% of 2-O-acetyl derivative *XVII*. For methyl-3-acetamido-4-O-acetyl-3,6-dideoxy-β-D-talopyranoside (*XVIII*) the following ^{1}H-NMR data were obtained: 1·22 (3 H, d, $J_{5,6}$ = 6·3, CH_3CH), 1·99 (3 H, s, CH_3. .CONH), 2·17 (3 H, s, CH_3COO), 3·60 (3 H, s, CH_3O), 6·32 (1 H, d, $J_{3,NH}$ = 8·0, NH), 5·17 (1 H, q, $J_{4,5}$ = 1·1, $J_{3,4}$ = 3·2, $H_{(4)}$), 4·40 (1 H, d, $J_{1,2}$ = 1·2, $H_{(1)}$), 4·23 (1 H, m, $J_{3,NH}$ = 8·0, $J_{2,3}$ = 3·2, $J_{3,4}$ = 3·2, $H_{(3)}$), 3·66 (1 H, o, $J_{4,5}$ = 1·1, $J_{5,6}$ = 6·3, $H_{(5)}$), 3·8—3·5 ($H_{(2)}$).

Deacetylation of Di-O-acetyl Derivative *X*

A mixture of 150 mg of 2,4-di-O-acetyl derivative *X* (0·495 mmol), 4·5 g of alkaline alumina and 8 ml of benzene were allowed to stand at room temperature with occasional shaking for 26 hours. Alumina was filtered off, washed with 80 ml of a mixture of benzene and ethanol 3 : 1 and the combined filtrates were evaporated. Chromatography on 20 g silica gel with benzene–ethanol 100 : 5 gave 95 mg (0·314 mmol, 63·3%) of the starting compound *X*, 5 mg (0·02 mmol, 3·9%) of 2-O-acetyl derivative *XI*, 27 mg (0·104 mmol, 20·9%) of 4-O-acetyl derivative *XII* and 11 mg (0·05 mmol, 10·4%) of acetamidogalactoside *I*.

Methyl 3-Acetamido-2-O-acetyl-3,6-dideoxy-4-O-methanesulfonyl-β-D-galactopyranoside (*XIV*)

0·05 ml of methanesulfonyl chloride was added at −70°C to a solution of 70 mg of 2-O-acetyl derivative *XI* in 2 ml of pyridine and the mixture was allowed to stand at −16°C for 48 hours. After decomposition with water the mixture was evaporated and purified by filtration through a column of 8 g of silica gel in benzene–ethanol 100 : 2. The obtained 85 mg (93%) of compound *XIV* were crystallized from a mixture of ethyl acetate–light petroleum; m.p. 196—198°C, $[\alpha]_D$ ±0°, $[\alpha]_{365}$ +10° (chloroform). ^{1}H-NMR data: 1·31 (3 H, d, $J_{5,6}$ = 6·5, CH_3CH), 1·94 (3 H, s, CH_3CONH), 2·06 (3 H, s, CH_3COO), 3·13 (3 H, s, CH_3SO_2O), 3·49 (3 H, s, CH_3O), 3·85 (1 H, o, $J_{4,5}$ = 0·7, $J_{5,6}$ = 6·5, $H_{(5)}$), 4·40 (1 H, d, $J_{1,2}$ = 7·7, $H_{(1)}$), 4·86 (1 H, q, $J_{1,2}$ = = 7·7, $J_{2,3}$ = 11·0, $H_{(2)}$), 4·34 (1 H, m, $J_{3,NH}$ = 8, $J_{2,3}$ = 11·0, $J_{3,4}$ = 2,6, $H_{(3)}$), 4·86 (1 H, q, $J_{3,4}$ = 2·6, $J_{4,5}$ = 0·7, $H_{(4)}$), 6·12 (1 H, d, $J_{3,NH}$ = 8, NH). For $C_{12}H_{21}NO_8S$ (339·4) calculated: 42·47% C, 6·24% H; found: 42·43% C, 6·28% H.

Methyl 3-Acetamido-4-O-acetyl-3,6-dideoxy-2-O-methanesulfonyl-β-D-galactopyranoside (*XIII*)

A mixture of 64 mg of 4-O-acetyl derivative *XII*, 2 ml of pyridine, and 0·05 ml of methanesulfonyl chloride was allowed to react in the same manner as described for the preparation of substance *XIV*. After working up 75 mg (93%) of substance *XIII* were obtained, which were identical with the described preparation[9]; m.p. 179—180°C, $[\alpha]_D$ +20·5° (chloroform).

Methyl 2-Acetamido-3,4-di-O-acetyl-2,6-dideoxy-β-D-idopyranoside (*V*)

A mixture of 3·6 g of compound *XVI*, 1·5 mg of sodium acetate trihydrate, 6 ml of water and 100 ml of 2-methoxyethanol was refluxed for 72 hours (see[9]). The solvents were evaporated, the residue was dried by repeated evaporation with toluene and acetylated with 15 ml of acetic anhydride and 30 ml of pyridine at room temperature. After decomposition with water and evaporation the residue was chromatographed on a column of silica gel (30 g) with benzene–ethanol mixture (100 : 5). In addition to 2·17 g (89%) of compound *IV* 120 mg (5%) of derivative *V* were obtained which had higher R_F value; two additional minor components differed in their R_F values from methyl 3-acetamido-2,4-di-O-acetyl-3,6-dideoxy-β-D-galactopyranoside (*X*). Derivative *V* was crystallized from a mixture of ethyl acetate–light petroleum, m.p. 140—144°C (under sublimation), $[\alpha]_D$ —37° (chloroform). ^{1}H-NMR data: 1·26 (3 H, d, $J_{5,6}$ = 6·5, CH_3CH), 1·99 (3 H, s, CH_3CONH), 2·10 (6 H, s, CH_3COO), 3·50 (3 H, s, CH_3O), 5·95 (1 H, d, $J_{2,NH}$ = 9·0, NH), 5·13 (1 H, t, $J_{2,3} = J_{3,4}$ = 5·1, $H_{(3)}$), 4·88 (1 H, q, $J_{3,4}$ = 5·1, $J_{4,5}$ = 3·1, $J_{2,4}$ = 0·5, $H_{(4)}$), 4·69 (1 H, d, $J_{1,2}$ = 2·5, $H_{(1)}$), 4·25 (1 H, m, $J_{1,2}$ = 2·5, $J_{2,3}$ = 5·1, $J_{2,NH}$ = 9·0, $J_{2,4}$ = = 0·5, $H_{(2)}$), 4·14 (1 H, o, $J_{4,5}$ = 3·1, $J_{5,6}$ = 6·5, $H_{(5)}$). For $C_{13}H_{21}NO_7$ (303·3) calculated: 51·48% C, 6·98% H, 4·62% N; found: 51·28% C, 7·12% H, 4·66% N.

Methyl 2-Acetamido-2,6-dideoxy-β-D-idopyranoside (*IX*)

3,4-Di-O-acetyl derivative *V* (60 mg) was dissolved in 5 ml of methanol, a drop of 1M sodium methoxide solution was added and the mixture allowed to stand at room temperature for 2 hours. After elimination of the sodium ions with Amberlite IR-120 (H^+) and evaporation of methanol the product (40 mg, 92%) was crystallized repeatedly from ethyl acetate–light petroleum; m.p. (after repeated crystallizations) 149—153°C, $[\alpha]_D$ —108° (water). For $C_9H_{17}NO_5$ (219·2) calculated: 49·31% C, 7·82% H; found: 49·18% C, 7·88% H.

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