OXIDATION OF COMPLETELY METHYLATED METHYL GLYCOPYRANOSIDES BY CHROMIC ANHYDRIDE

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Chromic anhydride in AcOH readily oxidizes completely acetylated hexopyranosides with an axial hydrogen atom on C_1 into esters of 5-ketoaldonic acids, while the anomeric hexopyranosides with an equatorial hydrogen atom at C_1 are stable and are converted only to a small extent to the respective 1-O-acyl derivatives [1, 2]. These differences in the oxidation rates and the reaction products have been used to establish the configuration of the glycoside linkages in the acetates of oligo and polysacchardies [3] and to selectively hydrolyze polysaccharides into oligosaccharide fragments [4, 5].

It seemed interesting to extend this approach to methylated polysaccharides. In the cases of selective oxidation, it could be possible to obtain information both on the configuration of the glycoside linkages and on their position. The data available in the literature on the oxidation of methylated hydrocarbons by CrO_3 are contradictory. On the one hand, it is known that they are unstable with respect to the action of CrO_3 and are converted into the corresponding O-formula derivatives [2]. On the other hand, the oxidation of methyl 2,3,4-tri-O-methyl-β-D-glucopyranoside yields the corresponding uronic acid with a 77% yield [6]. Therefore, we could expect to find reaction conditions under which the oxidation at the anomeric centers with the respective configuration would proceed considerably more rapidly and completely than the undesirable side reactions. For this purpose, we carried out a detailed investigation of the oxidation of methyl 2,3,4,6-tetra-O-methyl-β-D-galactopyranoside (I).

It turned out that the oxidation of I by CrO_3 proceeds very satisfactorily in AcOH at 20-25° and in the presence of 3-4 equivalents of the oxidizing agent. At lower temperatures (0-10°) the oxidation rate becomes negligibly small. Under the optimum conditions found, I is oxidized practically completely in 1-2 h, and the reaction mixture consists mainly of three compounds. When the reaction time is increased to 5-6 h, the mixture is found (by gas — liquid chromatography) to contain four main reaction products (II-V), whose structures have been established mainly with the aid of mass spectrometry (Table 1) and confirmed by other methods in individual cases.

When the reaction was carried out in the presence of dulcitol hexaacetate as an internal standard, the total yield of volatile reaction products was > 90% according to the gas — liquid chromatographic data. At first only compounds III-V, the amounts of which (especially of IV and V) gradually increase, are found in the mixture. Compound II appears after 3-4 h. The last compound proved to be dimethyl L-threo-2,3-dimethoxysuccinate.† The principal fragments in the mass spectra fit those expected for the structure indicated, i.e.,

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†The configurations of the centers have been assigned on the basis of the structure of the original glycoside.

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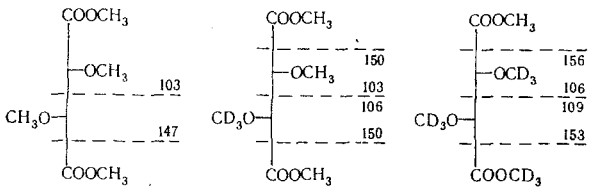
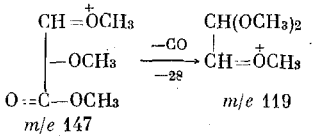


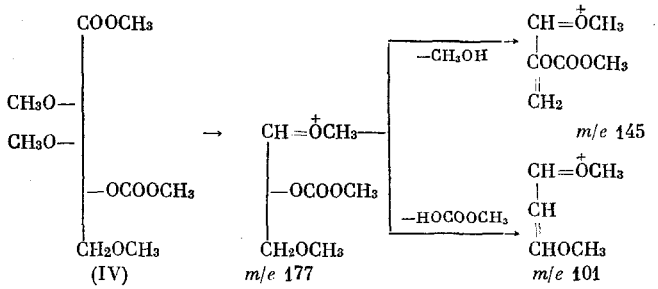
The ion with m/e 147 decomposes further to form an ion with m/e 119 according to the scheme



According to the mass-spectrometric data, the reduction of II by $NaBH_4$ followed by acetylation produces the respective polyols. The oxidation of the deuterated analogs of I containing trideuteromethyl groups in positions 3 (I-3) or 2, 3, 4, and 6 (I-2, 3, 4, 6) yield IIa and IIb with one or three CD_3 groups, respectively, according to their mass spectra. Thus, II is formed from the C_1C_4 fragment of the original compound (I).

Compound III is 2,3,4,6-tetra-O-methyl-D-galactono-1,5-lactone: Its mass spectra and retention time coincide completely with the mass spectrum and retention time of a known sample, and the mass spectrum of the polyol obtained when it is reduced and subsequently acetylated coincides with the mass spectrum of 2,3,4,6-tetra-O-methyl-1,5-di-O-acetyldulcitol [7].

Compound IV is clearly methyl 2,3,5-tri-O-methyl-4-O-carboxymethyl-D-lyxonate. The principal ions in the mass spectrum of IV form as a result of the cleavage of the $C_2 - C_3$ bond with localization of the charge on the $C_3 - C_5$ fragment followed by the splitting off of a CH_3OH or CH_3OCOOH molecule according to the scheme



The mass spectra of the labeled analogs of IV obtained from labeled analogs of I containing CD_3O groups in positions 1, 3, 6; 3 and 4; 2 and 6; and 2, 3, 4, and 6 display the corresponding displacements in these peaks.* The reduction of IV by $NaBH_4$ followed by acetylation yields 2,3,5-tri-O-methyl-1,4-di-O-acetylpentitol, as indicated by the gas — liquid chromatographic data and mass spectra of the latter [7]. The PMR spectrum of IV has three singlets (δ, ppm), viz., 3.33 (3H), 3.40 (3H), and 3.45 (3H), whose chemical shifts are characteristic of methoxy groups and methylated monosaccharides [8], and a signal at 3.8 ppm (6H), whose position is characteristic of methoxy groups in methyl esters of carboxylic acids [9].

Compound V is 2,3-di-O-methyl-4-O-carboxymethyl-D-lixosaccharic acid. The principal fragments in the mass spectrum of V are shifted 14 mass units toward higher masses from those in the mass spectrum of IV. This corresponds to the replacement of the two C_5 hydrogen atoms by oxygen and the formation of a second carbomethoxy group. The labeled analogs of I containing CD_3O groups in the positions listed above yielded derivatives of V whose mass spectra show shifts in the peaks for these fragments, which are consistent with the proposed structure. The reduction of V by $NaBH_4$ followed by acetylation produces 2,3-di-O-methyl-1,4,5-tri-O-acetylpentitol, according to the gas — liquid chromatographic data. The structure of this compound follows unambigously from its mass spectrum [7].

On the basis of the data presented, a general scheme for the oxidation of I by CrO_3 can be represented in the following manner

*A detailed investigation of the mass spectra will be published separately.

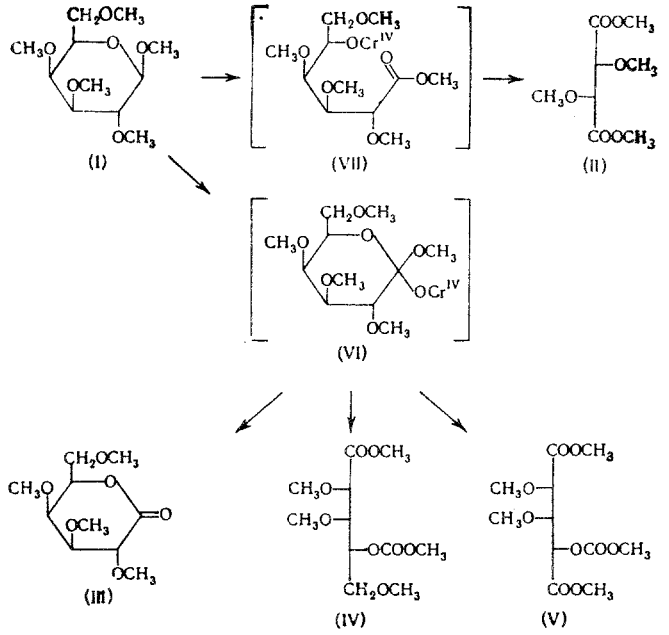
TABLE 1. Principal Products of the Oxidation of Methyl 2,3,4,6-Tetra-O-methyl-β-D-galactopyranoside (I) by CrO_3 in AcOH (20°, 6 h)

Compound	Retention time *	Relative yield†	Mass spectrum m/e, % ‡
(II)	0,5	30	206(2), 174(6), 147(14), 119(58), 118(18), 117(19), 103(100), 88(19), 85(12), 75(25), 73(38), 59(15), 45(25)
(III)	1,2	14	234(7), 145(100), 129(4), 113(17), 102(20), 101(62), 88(84), 85(33), 75(30), 73(30), 72(42), 71(33), 45(80)
(IV)	1,4	39	221(0,5), 177(50), 145(93), 119(24), 101(100), 89(17), 88(79), 87(24), 85(13), 75(57), 73(30), 71(24), 59(50), 45(170)
(V)	1,6	14	191(60), 173(16), 159(120), 149(14), 147(10), 145(22), 119(38), 118(50), 117(18), 115(23), 113(12), 103(60), 101(100), 89(20), 88(30), 87(17), 75(170), 73(37), 71(24), 59(36), 45(200)
Other	—	3	

*Relative to the original glycoside, 5% NPHS, 150°.

†The total yield of volatile reaction products was taken as 100%.

‡For the conditions see the Experimental part.



Obviously, the initial attack of the oxidizing agent is directed at the anomeric center and results in the formation of a hypothetical intermediate compound (VI) [10, 11]. This compound may decompose with the cleavage of one of the following three bonds: $C_1 - O_1$ (which results in the formation of lactone III); $C_1 - O_5$ [which results in the formation of VII, which is subsequently converted to a derivative of tartaric acid (II)]. As will be shown below, depending on the substituent at C_5 in pyranoside, this intermediate compound can also be converted into derivatives of 5-ketoaldonic acid, which are analogous to those obtained from the acetates of methyl β-D-hexopyranosides. Finally, compound VI may decompose with cleavage of the $C_1 - C_2$ bond (in the case of I, this is the main decomposition path and produces IV and V).

It is interesting to note that the oxidation of CH_3O groups into formyl groups was not observed at all, nor was the formation of methyl 2,3,4,6-tetra-O-methyl-L-arabinohexulos-5-onic acid, which might have been expected in analogy to the oxidation of the acetates of the hexopyranosides [1, 2]. At the same time, a very substantial portion of the reaction products corresponded to derivatives IV and V, which were not among the products of the oxidation of acetates of monosaccharides in [1, 2]. It should also be noted that we could not convert an isolated sample of IV into V by oxidation. This suggests that V is possibly formed from I as a result of the oxidation of the latter into a derivative of D-galactouronic acid followed by the cleavage of the $C_1 - C_2$ bond.

TABLE 2. Oxidation Products of Methyl 2,3,4-Tri-O-methyl-β-D-fucopyranoside by CrO_3 in AcOH (20°, 2 h)

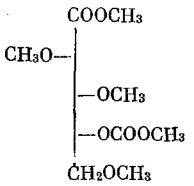
Compound	Retention time	Relative yield	Mass spectrum m/e, %
(XVII)	1,25	18	204(4), 131(6), 115(33), 101(25), 88(100), 85(13), 75(58), 73(27), 72(21), 45(19)
(XVIII)	2,2	26	191(10), 159(7), 147(100), 103(70), 101(18), 88(55), 85(17), 75(48), 73(40), 71(60), 45(70)
(XIX)	1,3	56	191(12), 175(2), 159(43), 147(15), 143(10), 131(10), 119(33), 103(26), 101(100), 88(44), 85(13), 75(56), 73(22), 59(26), 45(48), 43(93)

Inasmuch as CrO_3 in AcOH is used for the selective oxidation of the glycoside linkages, processes producing II and III are undesirable, since a glycoside linkage is destroyed during the formation of these compounds. This results in the degradation of the aglycon and, in the case of a polysaccharide, in the degradation of the next monosaccharide unit in the chain. Conversely, during the formation of IV and V, a glycoside linkage is replaced by an esteric linkage, which protects the aglycon from the oxidizing agent, but it can subsequently easily be degraded under conditions which rule out the cleavage of the glycoside linkages when the anomeric centers are resistant to oxidation.

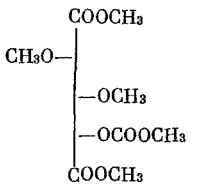
Thus the problem consisted of finding the conditions under which the yield of compounds IV and V would be maximal, and the formation of II and III would be reduced to a minimum. We succeeded in showing that the best results are provided by the oxidation of I by 2-4 equivalents of CrO_3 at 20-25° for 1-2 h in Ac_2O. Under these conditions I is oxidized practically completely. According to the data from gas — liquid chromatography with an internal standard (dulcitol hexaacetate), the total yield is 90-95%, and the amounts of II and III are insignificant (2-3%). In the present case Ac_2O has an important advantage over AcOH as a solvent. The formation of lactone III is observed in it. In addition, CrO_3 dissolves completely, and the reaction proceeds in a homogeneous medium. This offers great practical conveniences.

The next stage involved ascertaining the nature of the relationship between the structure and the configuration of the methylated methyl glycoside and the composition of its oxidation products. For this purpose, we subjected methyl 2,3,4,6-tetra-O-methyl-β-D-glycopyranoside (VIII), methyl 2,3,4-tri-L-methyl-β-D-fucopyranoside (IX), and α (X) and β (XI) anomers of methyl L-arabopyranoside, and the completely methylated α anomers of D-glucopyranoside (XII), D-galactopyranoside (XIII), and D-mannopyranoside (XIV) to oxidation under the optimum conditions we found.

The oxidation of VIII under the conditions described above, like the oxidation of I, yields methyl 2,3,5-tri-O-methyl-4-O-carboxymethyl-D-arabonate (XV), whose mass spectrum is identical to the mass spectrum of IV, as the main product. The yield of XV was 82%. Besides XV, dimethyl 2,3-di-O-methyl-4-carboxymethyl-D-arabosaccharate (XVI) was found in the reaction product (10% yield). Its mass spectrum coincided completely with the mass spectrum of V. The structural formulas of XV and XVI are

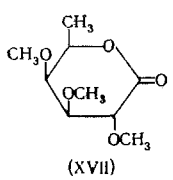


(XV)

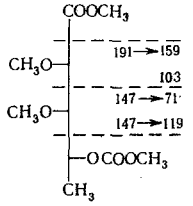


(XVI)

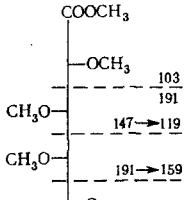
The oxidation of fucoside IX either in AcOH or in Ac_2O produced a somewhat different result and resulted in the formation of three main products, viz., XVII-XIX (Table 2)



(XVII)



(XVIII)



(XIX)

TABLE 3. Products of the Oxidation of Methyl 2,3,4-Tri-O-methyl-α-(X) and β-L-arabopyranoside (XI) by CrO_3 (20°, 2 h)

Compound	Retention time	Relative yield, % *	Mass spectrum m/e, %
(X) or (XI)	1	30 (30) 15 (5)	—
(XX)	0,6	5 (3) 5 (4)	—
(XXI)	1,2	10 (7) 20 (20)	See [13]
(XXII)	1,3	20 (30) 50 (50)	177(7), 147(1), 146(3), 145(4), 133(100), 119(23), 89(56), 88(17), 85(17), 75(18), 74(11), 73(32), 71(13), 45(67), 44(44), 43(50)
(XXIII)	1,4	35 (10) 10 (6)	191(6), 177(3), 147(45), 133(100), 131(22), 101(53), 99(10), 89(22), 88(40), 87(37), 73(37), 71(27), 70(50), 59(31), 45(22), 44(16), 43(62)
Other	—	5 (20) — (15)	—
(XXIV)	—	— —	191(12), 147(94), 119(100), 115(20), 104(20), 103(20), 101(14), 88(34), 85(20), 75(65), 73(81), 59(20), 45(60)

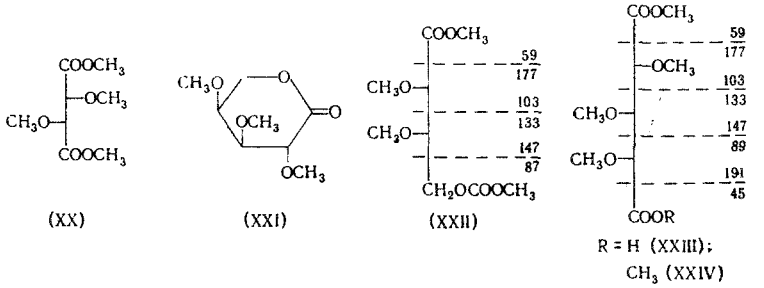
*The amounts of the compounds obtained when the oxidation reaction was carried out with Ac_2O are indicated in parentheses.

According to its mass spectrum, compound XVII is 2,3,4-tri-O-methyl-D-fucono-1,5-lactone. As on the case of galactonolactone III, the mass spectrum of XVII contains a small peak of a molecular ion (m/e 204) and peaks with m/e 115 (m/e 145 in the case of III) and m/e 72, which are characteristic of the 1,5-lactones of the aldonic acids. According to the mass-spectrometric data, the reduction of XVII by $NaBH_4$ followed by acetylation yields 6-deoxyhexitol [7]. Compound XVIII is methyl 2,3-di-O-methyl-4-O-carboxymethyl-6-deoxy-D-lixonate. Its mass spectrum is similar to the mass spectrum of IV with the one difference that all the peaks of the ions which contain C_6 are shifted 30 mass units toward smaller masses owing to the absence of the CH_3O at C_6. The reduction and subsequent acetylation of XVIII yield the corresponding 5-deoxypentitol [7].

Compound XIX is methyl 2,3,4-tri-O-methyl-6-deoxy-L-arabinulos-5-onate. The nature of its mass spectrum is in good agreement with this structure, and its reduction and subsequent acetylation yields two 6-deoxyhexitols [7].

Thus, in the case of ducoside IX, a significant amount of 5-ketoaldonic acid (XIX), which is a derivative characteristic of the oxidation of hexopyranoside acetates [1, 2], forms along with the usual reaction products for methyl β-D-hexopyranosides, viz., XVII and XVIII. The formation of XIX is clearly due to the absence of a CH_3O group at the C_6 of the pyranoside.

The oxidation of methyl 2,3,4-O-methyl-α-(X) and β-L-arabopyranoside (XI) either with AcOH or with Ac_2O results in the formation of practically the same reaction products (XX-XXIII), although they form in slightly different ratios (Table 3). This can clearly be attributed to the appreciable content of the conformer in the $_1C^4 \rightleftharpoons {}^1C_4$ equilibrium with an axial proton at C_1 (which is capable of reacting with CrO_3) in the case of either of these anomers [12]. These products have the structures



(XX) (XXI) (XXII)

R = H (XXIII); CH_3 (XXIV)

Compound XXI is a lactone of 2,3,4-tri-O-methyl-arabonic acid [13] (mass spectrum). Compound XXII is methyl 2,3-di-O-methyl-4-methoxycarbonyl-L-erythronate. As in the case of IV, the cleavage of the $C_2 - C_3$ bond with localization of the charge on C_3 is most characteristic of this compound. According to the mass-spectrometric data, the reduction and subsequent acetylation of compounds XX-XII yield the respective polyols [7]. Compound XXIII is a monomethyl 2,3,4-tri-O-methyl-L-arabonate. Treatment of the reaction mixture by an ethereal solution of dizaomethane produces the dimethyl ester of this acid (XXIV), and its reduction and subsequent acetylation produces the corresponding polyol [7].

TABLE 4. Oxidation Products of Methyl α-D-Glycopyranosides XII-XIV in AcOH (20°, 6 h)

Compound	Relative amount, % of the total				
	position of the O-acyl group				
	original	6	4	3	unidentified compounds
(XII)	24	53	—	10	13
(XIII)	45	37	5	5	8
(XIV)	31	50	—	8	11

A comparison of the structures and composition of the oxidation products reveals that arabinopyranosides X and XI occupy an intermediate position between methyl β-D-hexopyranosides I and VIII, on the one hand, and methyl β-D-fucopyranoside IX and the acetates of the methyl β-D-hexopyranosides [1, 2], on the other hand. While derivatives of the aldopentanoic acids are dominant among the products of the former, the formation of considerable amounts of methyl 5-ketoaldonates is observed in the case of the latter. In the case of arabinopyranosides X and XI, both types of oxidation products are present in comparable amounts. It is thus clear that the presence and nature of the substituent at the C_5 of the pyranose ring has a decisive effect on the direction of the decomposition of the initially formed intermediate compound (VI).

In order to conclusively resolve the question of the possibility of the selective hydrolysis of methylated polysaccharides, it was necessary to ascertain how the most common methyl α-D-hexopyranosides (XII, XIII, and XIV) behave under the conditions selected. The oxidation was first carried out in AcOH for 6 h under the action of 4 equivalents of CrO_3. The oxidized mixtures were reduced by $NaBH_4$ and acetylated in cases of the possible oxidation of the CH_3O group at C_1 with the formation of 1-O-formyl derivatives, which are difficult to distinguish from the original compounds by mass spectrometry. Such treatment should convert the 1-O-formyl derivatives into the corresponding acetates of the polyols, which are readily distinguished from the original compounds [7], and the oxidation of the other CH_3O groups should yield the acetates of the partially methylated methyl α-D-glycopyranosides, whose mass spectrometry has also been thoroughly developed [14].

According to Table 4, under comparatively severe oxidation conditions relatively large amounts of the original glycosides (24-25%) are maintained in the mixture. The main reaction products are 6-O-acetates, according to the mass-spectrometric data [14]. However, oxidation in Ac_2O, i.e., under the conditions most favorable for the oxidation of methyl β-D-glycopyranosides, results in the complete oxidation of the α anomers, and practically all the possible mono- and di-O-acyl derivatives of the methyl glycopyranosides are detected among the reaction products.

Thus, despite numerous experiments, we did not succeed in finding the conditions under which the methylated methyl β-glycopyranosides would be completely oxidized to the appropriate products, and the methyl α-glycopyranosides would remain basically unchanged. In light of the data obtained, the prospective use of CrO_3 for the selective oxidation of glycoside linkages in completely methylated oligo- and polysaccharides is generally not very encouraging. This approach may be recommended only for the oxidation of glucans which are linked by 1,6-glycoside linkages (oxidation in AcOH). In addition, the laws demonstrated in this work for the oxidation may serve as a basis for devising preparative methods for the direct oxidation of methylated methyl β-glycopyranosides to derivatives of lower sugars with cleavage of the $C_1 - C_2$ bond.

EXPERIMENTAL METHOD

The original methyl glycosides were synthesized according to known methods (see [13]). The purity of the compounds obtained was monitored by thin-layer chromatography on SiO_2, gas — liquid chromatography, and mass spectrometry. The mass spectra of the methylated derivatives of the glycosides and their oxidation products were recorded on a Varian MAT 111 Gnom instrument with the use of a column with 3% SE-30 and 3% ECNSS-3M on Chromosorb W. The gas — liquid chromatographic analyses were carried out on a Varian 1700 instrument in columns 1-2 m long with 5% NPHS, 5% SE-30, and 3% ECNSS-3M on Chromosorb W. The programmed variation in the temperature was from 80-100° to the maximum temperature for the particular phase, the carrier gas was nitrogen, and the rate was 30 ml/min. The IR spectra of the compounds were recorded on a UR-20 spectrometer in a CCl_4 solution, and the PMR spectra were recorded on a Varian Da-60-IL radiospectrometer.

Oxidation of Methylated Methyl Glycopyranosides by CrO_3 in Glacial AcOH. Freshly redistilled glacial AcOH in an amount equivalent to 1 ml of acid per 10 mg of CrO_3 was added to a weighed portion of CrO_3 (10-100 mg), which was preliminarily dried over P_2O_5. The mixture was stirred for 1 h at 20°. Afterwards an equal (by weight) amount of the methyl glucoside was added to it, and the mixture was

stirred for 1-2 h at 20-25°, aliquots being collected periodically. Each aliquot was diluted with an equal volume of water and thoroughly extracted with $CHCl_3$. The extract was washed with water, dried by calcined Na_2SO_4, filtered, and evaporated. The remaining product was investigated by gas — liquid chromatography and then by mass spectrometry.

Some of the compound was reduced by $NaBH_4$ (10 h), acetylated by Ac_2O in pyridine, and then investigated by the same methods. In the experiments in which dulcitol hexaacetate served as an internal standard, the yield of the reaction products was > 90% of the amount of the original compound.

Oxidation of Methylated Methyl Glycopyranosides by CrO_3 in Ac_2O. The oxidation of the methyl glycopyranosides was carried as in the preceding case. Freshly redistilled Ac_2O in an amount equivalent to 10 mg of the oxidizing agent per mg of the anhydride was added to a weighed portion of CrO_3 which was dried over P_2O_5. The mixture was stirred until the complete dissolution of the precipitate. Then an equal amount (by weight) of the methyl glycopyranoside was added, and the mixture was stirred for 1-2 h, aliquots being collected periodically. The subsequent operations were carried out as in the case described above.

The preparative oxidation of I was carried out in AcOH. The reaction products were isolated with the aid of column chromatography on SiO_2. The purity of the compounds was monitored by gas — liquid chromatography and mass spectrometry.

CONCLUSIONS

1. When CrO_3 in AcOH or Ac_2O acts on methylated methyl glycopyranosides with an axial proton at C_1, the glycoside center is initially subjected to the attack. The predominant direction of the subsequent course of the oxidation depends on the nature of the C_5 substituent on the pyranose ring: The oxidative splitting of the $C_1 - C_2$ bond is characterixtic of hexosides, and the oxidative splitting of the $C_1 - O_5$ bond is characteristic of the 6-deoxyhexosides and pentopyranosides.

2. In the case of the oxidation of methyl α-D-hexopyranosides under the same conditions, the attack of the CrO_3 is directed at the CH_3O groups. This oxidizes them to formyl groups. The group at C_6 is preferentially oxidized in AcOH, while a mixture of all the possible mono- and di-O-formyl derivatives forms in Ac_2O.

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