OXIDATION OF THE METHYL-3,6-ANHYDRO-D-GALACTOPYRANOSIDE ACETATES AND AGAROSE BY CHROMIC ANHYDRIDE

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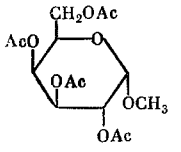
UDC 542.943:547.455

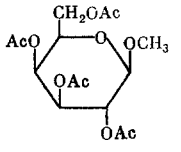
Reports that CrO_3 in AcOH solution can be used to carry out the selective oxidation of acetylated hexopyranosides [1, 2] have been followed by various attempts to determine glycoside bond configurations in oligo- and polysaccharide acetates by oxidations of this type [3]. The procedures in question here rest on the fact that CrO_3 readily oxidizes acetylated pyranosides with an axial hydrogen at the C^1 atom to 5-ketoaldonic acid esters while leaving pyranosides carrying an equatorial hydrogen at the C^1 atom unaffected. Although the oxidation of the acetylated hexofuranosides proceeds less selectively [2], conditions can be found under which the α-anomer, but not the β-anomer, will be oxidized [4]. Ac_2O, a compound in which both CrO_3 and the polysaccharide acetates are readily soluble, has been proposed for use as the solvent in work with the polysaccharides [5].

The present work was a study of the reactions of the anomeric acetates of the methyl-3,6-anhydrogalactopyranosides with CrO_3, and the behavior of polysaccharides containing a 3,6-anhydrogalactose residue under this type of oxidation. The action of CrO_3 in Ac_2O on methyl-2,3,4,6-tetra-O-acetyl-α- (I) and methyl-2,3,4,6-tetra-O-acetyl-β-D-galactopyranosides (II), methyl-2,4-di-O-acetyl-3,6-anhydro-α- (III) and methyl-2,4-di-O-acetyl-3,6-anhydro-β-D-galactopyranosides (IV), and agarose acetate (V) was investigated here. The galactosides (I) and (II) were used to determine the conditions required for carrying out the modified oxidation reaction. Since compounds (III) and (IV) show a $_4C^1$ conformation of the pyranose ring, and this conformation is reinforced by the presence of an additional ring, it was to be anticipated that the α-anomer (III) would be preferentially oxidized. Yields of the oxidation products, and the amounts of reactants remaining at the end of the reaction, were determined by gas–liquid chromatography, using the hexaacetate of inositol as an internal standard [6]. The structures of the reaction products were largely determined by chromatographic mass spectrometry (CMS).

The temperature proved to be the decisive factor in determining the degree of oxidation of (I). Thus 85-90% of the compound remained unoxidized at the end of a 15-min reaction at 0°C, and 80-83% unoxidized at the end of a similar reaction at 20°C; at the end of a 1.5-h oxidation at 50°C, the conditions recommended for polysaccharide oxidation in Ac_2O, only 22% of the compound (I) remained unoxidized [5]. Oxidation products could not be detected in a chloroform extract of the reaction mixture. The glucoside (II) was completely converted to the methyl ester of 2,3,4,6-tetra-O-acetyl-5-ketoaldonic acid (VI) by a 15-min oxidation at 20°C [2], the yield in separation being 55%. In other words, the selectivity of oxidation of(I) and (II) is maintained in Ac_2O, at least if reaction is carried out at elevated temperature.



(I)



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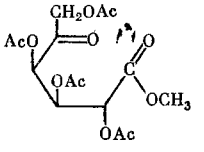

(VI)

A 15-min oxidation left 27% of (IV) unreacted at 20°C, while a 20-min oxidation at the same temperature left 3% of the compound unreacted. Here the oxidizing action was directed toward both the C^1 and C^6 atoms of the glycoside. The γ-lactone of methyl-2,4-di-O-acetyl-β-D-galactopyranosideuronic acid (VII) was obtained by extracting the reaction mixture with chloroform. A maximum lactone yield of ~10% was obtained in a 30-min reaction at 20°C. The structure of (VII) was deduced from mass spectrometry and confirmed by the following reaction sequence:

N. D. Zelinskii Institute of Organic Chemistry, Academy of Sciences of the USSR, Moscow. Translated from Izvestiya Akademii Nauk SSSR, Seriya Khimicheskaya, No. 2, pp. 394-399, February, 1980. Original article submitted December 12, 1978.

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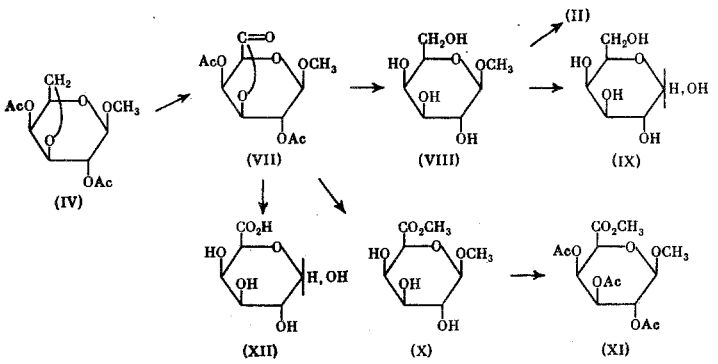
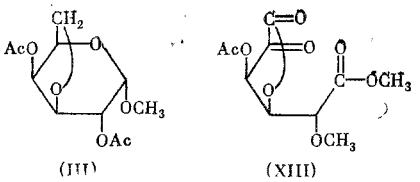


Methyl-β-D-galactopyranoside (VIII), prepared by reducing (VII) by $NaBH_4$ in 50% methanol solution, could be hydrolyzed in 2 N H_2SO_4 to give the galactose (IX), or acetylated to give compound (II). Treatment of compound (VII) with MeONa in methanol solution gave the methyl ester of methyl-β-D-galactopyranosideuronic acid (X), whose structure was determined from mass spectroscopy of the acetate (XI). Finally, hydrolysis of the lactone with 2 N H_2SO_4 led to the formation of D-galactouronic acid (XII), which was identified chromatographically.

The oxidation of (III) proceeded exothermically, leading rapidly to extensive destruction of the monosaccharide. Less than 1% of the compound remained unreacted at the end of a 15-min oxidation at 20°C, while complete consumption was attained in a 30-min oxidation at this same temperature. Traces of compounds of indeterminate structures were detected in the chloroform extract obtained in the standard treatment described in the Experimental section. Traces of compound (XIII), a derivative of ketogalactaric acid (CMS), were obtained by working at temperatures in the −30 to −40°C range and rapidly diluting the reaction mixture with cold toluene.

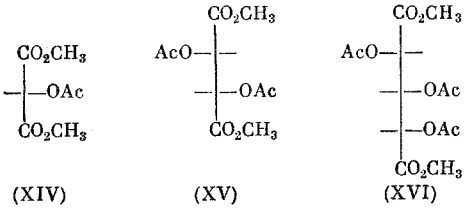


It could be reasonably assumed that this compound appears as an intermediate in the strong oxidation of both (III) and (IV).

From this it followed that the configuration of the glycoside center must affect the rate of oxidation of both (III) and (IV), the β-anomer being the more stable of the two compounds, just as anticipated. Both anomers are, however, almost completely broken down, the C^6 atom being more readily oxidized than the anomeric center in the 3,6-anhydro-galactopyranosides.

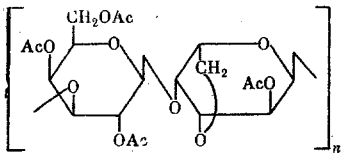
Analysis of the strong oxidation products was carried out by diluting the reaction mixture with a large amount of methanol and then evaporating it to dryness. This procedure assured that the excess CrO_3 would be consumed in oxidizing the alcohol [7]. Gas−liquid chromatography showed that some 90% of (I) was left unreacted at the end of a 15-min oxidation at 20°C, and this without the appearance of side products in the reaction mixture. Acid methanolysis followed by acetylation without preliminary removal of the chromic salts left the ratio of total galactosides to inositol acetate unaltered.

Proceeding in a similar manner, the reaction mixture remaining from the oxidation of (III) was diluted with methanol, evaporated, and then subjected to successive methanolysis and acetylation. Gas−liquid chromatography showed the principal reaction product components to be the dimethyl esters of acetyltartronic acid (XIV), di-O-acetyltartaric acid (XV), and tri-O-acetylarabinaric acid (XVI). The structures of these compounds were deduced from gas−liquid chromatography, and confirmed by comparison with the mass spectra of compounds of known structures in the cases of (XV) and (XVI). Compounds (XIV)-(XVI) were detected in products from the oxidation of (IV) but not (II).



It should be noted that there have been earlier reports of the appearance of tartaric acid among the products resulting from the oxidation of methylated glycosides by chromic anhydride [8].

On the basis of this preliminary work it was assumed that the oxidation of (V) would lead to the complete cleavage of the polymer with formation of a derivative of 5-ketoaldonic acid which would be, in turn, esterified by the products from oxidation of the 3,6-anhydro-L-galactose residue



(V)

Since the oxidation of (V) proceeds exothermically, the addition of CrO_3 must be carried out gradually. Carbohydrate fragments could not be detected in the organic phase resulting from extraction of the reaction products with chloroform [5]. Since dialysis of the reaction mixture led to an almost complete loss of the reaction products, it was assumed that reaction must have resulted in the extensive destruction of (V). Compounds (I), (II) and (VI), and (XIV)-(XVI), products resulting from the breakdown of 3,6-anhydro-L-galactose, were detected by gas–liquid chromatography and chromatographic mass spectroscopy of systems in which separation had been carried out by successive acid methanolysis and alkylation. Despite the fact that the galactose residues exist in the β-configuration, calculation showed that at least three such linkages remained unoxidized in each agarose acetate molecule (cf. [9]).

The attempt was made to detect the presence of fragments incorporating galactose residues joined by glycoside bonds with products resulting from oxidation of the 3,6-anhydro-L-galactose residues. This was done by treating solutions of the products from the oxidation of agarose acetate in methanol with $NaBH_4$. Chromatographic mass spectroscopy of systems which had been acetylated and chromatographed on silica gel showed only the presence of the polyol acetates (XVII)-(XX)

CH_2OAc	CH_2OAc	CH_2OAc	CH_2OAc
|	|	|	|
$CHOAc$	$(CHOAc)_2$	$(CHOAc)_3$	$(CHOAc)_4$
|	|	|	|
CH_2OAc	CH_2OAc	CH_2OAc	CH_2OAc
(XVII)	(XVIII)	(XIX)	(XX)

The indication here was that the glycoside bonds of the galactose residues remaining in the polysaccharide after oxidation are capable of cleaving under the action of bases. In fact, the pentaacetate of β-D-galactopyranose (XXI) was essentially the only product obtained by treating the products of oxidation of (V) with MeONa in methanol solution, with subsequent acetylation and chromatographing on silica gel, the yield being of the order of 30%, as calculated in terms of the galactose fragments in the agarose acetate molecule.

Thus, polysaccharide cleavage in the oxidation of (V) is largely the result of breakdown of the 3,6-anhydro-L-galactose residue to dicarboxylic acid derivatives. A considerable number of the D-galactose residues remain unoxidized, and this despite the fact that the glucoside centers exist in the β-configuration. Thus, conclusions concerning the configuration of the glucoside bonds in the molecule of (V) can give a distorted picture of polysaccharide structure if based on the results of oxidation studies alone.

EXPERIMENTAL

Descending paper chromatography (PC) was carried out on FN-15 paper, using the following solvent systems: tert-amyl alcohol–n-propanol–water (8 : 2 : 3, A); ethyl acetate–pyridine–water (2 : 1 : 2, B), n-butanol–pyridine–water (4 : 3 : 3, C); AcOH–n-butanol–water (4 : 1 : 1, D). IO_4^- and $AgNO_3$ were used for zone development, in general, and aniline phthalate in the case of the reducible saccharides. Thin-layer chromatography was carried out on unsupported KSK silica gel films, working in $CHCl_3$–acetone (9 : 1, E) and (1 : 1, F) systems. Concentrated H_2SO_4 was used for zone development, in general, and o-aminophenol–H_3PO_4 in the case of the 3,6-anhydro derivatives of the monosaccharides [10]. Gas–liquid chromatography was carried out on a Pye Unicam-104 system using a 150 × 0.6 cm column, the latter packed with 3% SE-30 for work at 200°C (G) and with 3% MGA for work at 220°C (I). Except in the case of compounds (VII) and (XXI), yields and retention times for the monosaccharide oxidation products were determined relative to inositol hexaacetate [6]. Peak areas and retention times were calculated with the aid of a Kent Chromalog-2 integrator. Chromatographic mass spectroscopy was carried out with a Varian MAT-111 system working at a 4 deg/min gradient over the interval from 130 to 300°C, and using a 150 × 0.2 cm column packed with 10% SE-30.

The galactosides (I) and (II) were prepared by the procedure of [11].

Methyl-2,4-di-O-acetyl-3,6-anhydro-α-D-galactopyranoside (III). The mixture obtained by adding 0.2 ml of Ac_2O to a solution containing 10 mg of methyl-3,6-anhydro-α-D-galactopyranoside dissolved in 0.2 ml of pyridine was evaporated with heptane and toluene in vacuum over a period of 24 h. TLC data showed an approximate 100% yield of product, R_f 0.6 (E), mp 82-83°C (from methanol), $[\alpha]_D$ +53° (C 1.3; $CHCl_3$). Mass spectra m/e: 229 (M − 31), 200 (M − 60), 157, 144, 127, 103, 99, 98, 85, 83, 43. Found: C, 51.01; H, 6.63%. $C_{11}H_{16}O_7$. Calculated: C, 50.77; H, 6.15%.

Methyl-2,4-di-O-acetyl-3,6-anhydro-β-D-galactopyranoside (IV). The preparation here was similar to that used in synthesizing methyl-3,6-anhydro-β-D-galactopyranoside. R_f 0.62 (E), mp 98-99°C (from methanol), $[\alpha]_D$ −85.4° (C 0.8; $CHCl_3$). The mass spectrum of (IV) was identical with that of (III). Found: C, 51.12; H, 6.28%. $C_{11}H_{16}O_7$. Calculated: C, 50.77; H, 6.15%.

General Method of Oxidizing Compounds (I)-(IV). A 30 mg sample of CrO_3 was added to a solution containing 10 mg of monosaccharide and 12 mg of inositol hexaacetate dissolved in 0.3 ml of Ac_2O. At the end of the reaction, the system was cooled, diluted with water, neutralized with $CaCO_3$ and $NaHCO_3$, and extracted with chloroform.

Oxidation of (I). The yields of compound (I) obtained in 15 min reaction were as follows: 85-90% at 0°C, 80-83% at 20°C; for a 90 min reaction at 50°C the yield was 22%. A mixture of (I) and the residue melted without a melting-point depression.

Oxidation of (II). A 15 min reaction at 20°C gave a 55% yield of the methyl ester of 2,3,4,6-tetra-O-acetyl-5-ketoaldonic acid (VI) [2]. Mass spectra (m/e): 317 (M − 59), 303 (M − 73), 275 (M − 59 − 42), 261 (M − 73 − 42), 243 (M − 73 − 60), 233, 219, 215, 201, 191, 183, 173, 157, 132, 115, 113, 103, 101, 73, 43.

Oxidation of (IV). The yield of residue (IV) at 20°C was 27% for a 15 min reaction and 3% for a 30 min reaction. The mass spectra of (IV) and its residue were identical. A 20 mg (10%) yield of the γ-lactone of 2,4-di-O-acetyl-β-methyl-D-galactopyranosideuronic acid was obtained from 0.2 g of (IV) by preparative TLC (30 min reaction at 20°C). R_f 0.7 (E). $[\alpha]_D$ −172° (C 0.2; $CHCl_3$), mp 112-113°C (from methanol). Mass spectra, m/e: 243 (M − 31), 232 (M − 42), 217, 201, 183, 171, 157, 154, 142, 141, 128, 115, 112, 103, 99, 43. Methyl-β-D-galactopyranoside (VIII), R_f 0.51 (A), was obtained by reducing (VII) with an excess of $NaBH_4$ (50% methanol, 20°C, 5 h); compound (II) was obtained by the acetylation of (VIII) (pyridine, Ac_2O, 20°C, 24 h). Hydrolysis of (VIII) (2 N H_2SO_4, 100°C, 4 H) gave the D-galactose R_{gal} 1 (C, D). Treatment of (VII) with MeONa in methanol solution (10 min, 20°C) gave the methyl ester of methyl-β-D-galactopyranosideuronic acid (X), R_f 0.3 (F), which was identified by mass spectrometry of the acetate (XI), R_f 0.55 (E). Mass spectra, m/e: 317 (M − 31), 289 (M − 59), 257 (M − 31 − 60), 246 (M − 102), 243, 229, 197, 187, 186, 169, 157, 145, 132, 115, 103, 43. Hydrolysis of (VII) (2N H_2SO_4, 80°C, 24 h) gave D-galactouronic acid, R_f 0.39 (B).

Oxidation of (III). a) The yield of residue (III) in 15 min reaction at 20°C was less than 1%; residue formation could not be detected in a 30 min reaction at this same temperature. When working over the interval from −30 to −40°C, the reaction mixture was diluted with cold toluene and washed with water at the end of 30 min. Gas−liquid chromatography disclosed the presence of traces of compound (XIII); this was assumed to have a γ-lactone ketogalactaric acid structure on the basis of the following mass spectrum, m/e: 246 (M − 42), 229 (M − 59), 215, 187, 186, 174, 157, 115, 85, 43.

b) The reaction mixture (20°C, 30 min) was carefully poured into absolute methanol, and the resulting solution evaporated in dryness. The dry residue was treated with a 1% solution of HCl in methanol (0.43 ml of AcCl dissolved in 10 ml of CH_3OH), held at the boiling point for 0.5 h, neutralized with $PbCO_3$, evaporated, acetylated, carried to the silica-gel-packed column (LS 40/100) and eluted with $CHCl_3$. Chromatographic mass spectroscopy showed the product to be a mixture of the dimethyl ester of O-acetyltartronic acid (XIV), m/e: 159 (M − 31), 148 (M − 42), 131 (M − 59), 104, 43; the dimethyl ester of di-O-acetyltartaric acid (XV), m/e: 231 (M − 31), 203 (M − 59), 189, 174, 161, 143, 132, 129, 119, 101, 99, 43; the dimethyl ester of tri-O-acetylaravinaric acid (XVI), m/e: 303 (M − 31), 275 (M − 59), 261, 233, 203, 201, 190, 173, 161, 145, 132, 90, 43. Compounds (XV) and (XVI) were identified from gas−liquid chromatography and study of the mass spectra of compounds of known structures.

Agarose Acetate (V). A 1 g sample of agarose was dissolved in the minimum amount of dry formamide; to this solution there were successively added, under constant stirring, 20 ml of Ac_2O and 20 ml of pyridine, and the mixture allowed to stand for 24 h at 20°C, and then poured into 1 liter of water. The resulting precipitate was washed with water and methanol, taken up in chloroform, and the resulting mixture evaporated to a thick syrup from which compound (V) was precipitated with methanol. Study of the IR spectra showed this compound (V) to be free of OH groups.

TABLE 1. Retention Times of the Monosaccharide Acetates and Oxidation Products, Relative to Inositol Hexaacetate

Column	(I)	(II)	(III)	(IV)	(VI)	(VII)	(XV)	(XVI)	(XIII)
G	0,60	0,60	0,26	0,26	0,60	0,32	–	–	0,30
I	0,45	0,54	0,20	0,20	>1	0,41	0,06	0,22	–

Oxidation of (V). To a solution containing 0.5 g of (V) dissolved in 15 ml of Ac_2O there was added 1.5 g of CrO_3, addition being made portionwise in the course of 1 h time. This mixture was allowed to stand until dissolution was completed and then carefully poured into anhydrous methanol, and the new mixture evaporated to dryness. The dry residue was then treated as described above (cf. Oxidation of (III), point b). Chromatographic mass spectrometry showed the product obtained here to be a mixture of (XIV)-(XVI), (I), (II), and (VI). The dry residue left from the oxidation of (V) was dissolved in anhydrous methanol and treated with an excess of $NaBH_4$ (20°C, 12 h). The resulting solution was neutralized with Ky-2 (H^+), evaporated, acetylated, carried over to the silica-gel-packed column, and eluted with chloroform. Chromatographic mass spectroscopy showed the product obtained to be a mixture of polyol acetates (XVII), m/e: 159 (M − 59), 158 (M − 60), 145, 116, 115, 103, 86, 73, 43; (XVIII), m/e: 231, 217, 175, 170, 157, 145, 128, 116, 115, 103, 86, 73, 43; (XIX), m/e: 289, 260, 243, 229, 217, 200, 187, 175, 158, 145, 127, 115, 103, 98, 85, 43; (XX), m/e: 375, 361, 289, 272, 259, 217, 187, 175, 170, 157, 145, 139, 128, 127, 115, 103, 97, 85, 43 [12].

The dry residue was treated with MeONa in methanol solution (10-15 min, 20°C). Further treatment was carried out in the manner described above. From 0.5 g of (V) there was obtained 0.15-0.17 g (~30%) of the pentaacetate of β-D-galactopyranoside (XXI), R_f 0.55 (E), mp 140-142°C (from methanol), $[\alpha]_D$ + 20.5° (C, 0.5; $CHCl_3$). Mass spectra, m/e: 331, 288, 275, 245, 242, 200, 187, 182, 169, 157, 145, 140, 127, 126, 115, 103, 98, 43; cf. [13, 14].

CONCLUSIONS

1. Treatment of the acetylated methyl-3,6-anhydrogalactopyranosides with chromic anhydride dissolved in acetic anhydride leads to gentle oxidation at the C^6 atom with subsequent formation of derivatives of the dicarboxylic acids. Formation of the γ-lactone of the methyl glycoside of galactouronic acid is observed in the case of the β-anomer.

2. A considerable number of the D-galactose residues remain unaffected during oxidation of the agarose acetate, and this despite the fact that the glycoside centers exist in a β-configuration. Polysaccharide cleavage is largely due to breakdown of the 3,6-anhydro-L-galactose residues.

3. A method for separating the products resulting from strong glycoside acetate oxidation by chromic anhydride is proposed.

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