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Oxidation of Carbohydrate Derivatives with Silver Carbonate on Celite. X. Identification of Three Mono-*O*-isopropylidene Derivatives of D-Galactose

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Treatment of D-galactose with anhydrous cupric sulfate in acetone and dimethylformamide gave three mono-*O*-isopropylidene derivatives, identified as 5,6-*O*-isopropylidene-D-galactofuranose (I), 3,4-*O*-isopropylidene-D-galactopyranose (II), and 4,6-*O*-isopropylidene-D-galactopyranose (III). The identification was based on oxidation of the *O*-isopropylidene-derivatives with silver carbonate on Celite; this oxidant gave in methanol *O*-isopropylidene derivatives of D-glyceraldehyde, D-lyxose, and D-threose, respectively, from I, II, and III, in addition to different esters of aldonic acids. Oxidation in benzene, or dimethylformamide and benzene, resulted in less extensive degradation of the *O*-isopropylidene-D-galactoses.

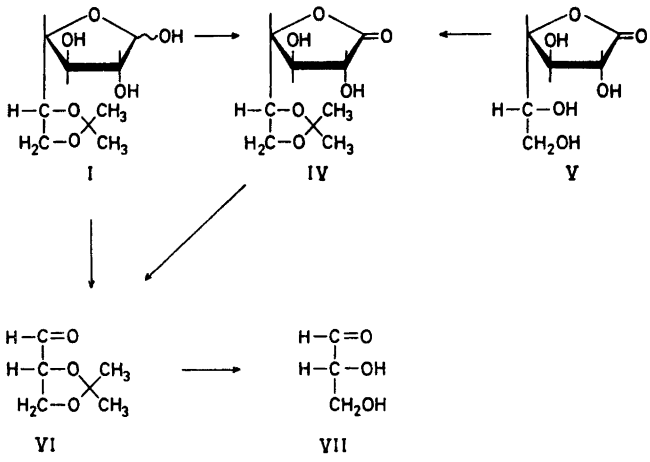
Silver carbonate on Celite has previously been shown to degrade aldoses stepwise from C-1 in methanolic solution. Compounds containing two or three carbon atoms are the final products obtained from unsubstituted aldoses,[1] whereas substituted sugars are degraded until the substituent prevents further shortening of the carbon chain.[2,3] These results suggested that the reagent might be of potential utility in location of substituents in aldoses, and in the present paper the application of the oxidant in identification of three reducing monoisopropylidene derivatives of D-galactose is described. Investigations of the applicability of the method on other types of substituted carbohydrates are in progress.

RESULTS AND DISCUSSION

Treatment of D-galactose with anhydrous cupric sulfate in acetone containing 20 % dimethylformamide at reflux temperature for 20 h, resulted in formation of three mono-*O*-isopropylidene derivatives. In addition, small amounts were also formed of two compounds which were chromatographically indistinguishable from 1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose and 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose. These derivatives were previously reported to be the products when D-galactose was treated with the same reagents for 2 days in the presence of greater proportions of acetone.[4]

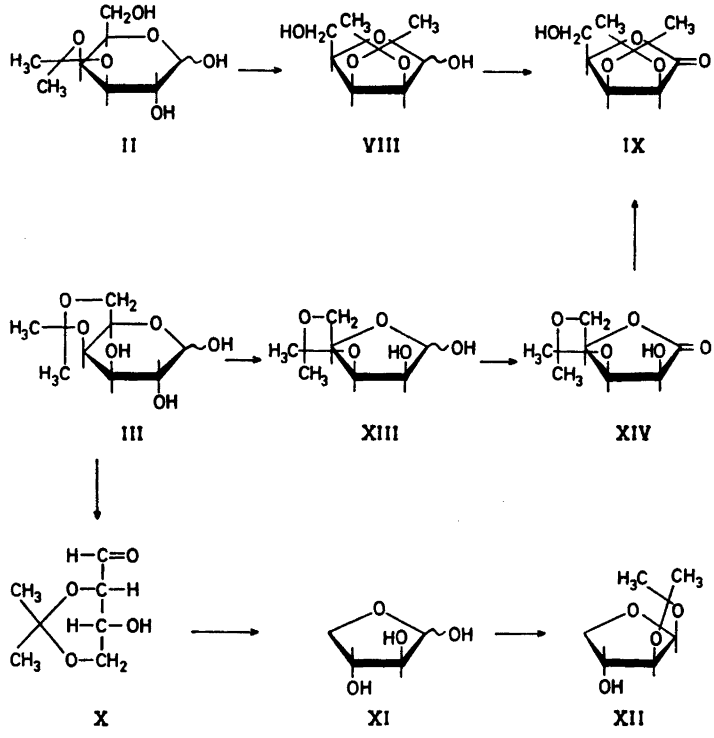
The mono-*O*-isopropylidene derivatives were separated on a silica gel column, and identified as 5,6-*O*-isopropylidene-D-galactofuranose (I), 3,4-*O*-isopropylidene-D-galactopyranose (II), and 4,6-*O*-isopropylidene-D-galactopyranose (III). The yields were 22, 15, and 13 %, respectively. The total amount of mono-*O*-isopropylidene derivatives was somewhat higher than the sum of the above yields since some fractions contained mixtures of I and II.

The identification of the products was based on oxidation with silver carbonate on Celite in methanol, benzene, or benzene and dimethylformamide. Oxidation of I in refluxing benzene gave 5,6-*O*-isopropylidene-D-galactono-1,4-lactone (IV). This compound was for comparison also prepared from D-galactono-1,4-lactone (V) with anhydrous cupric sulfate and acetone; the structure was established by infrared spectroscopy in combination with periodate oxidation at pH 7, which led to the formation of 2,3-*O*-isopropylidene-D-glyceraldehyde (VI). In methanolic solution, oxidation of I gave a product mixture



in which no aldotetrose, -pentose, or -hexose derivative could be detected; a compound chromatographically indistinguishable from 2,3-*O*-isopropylidene-D-glyceraldehyde (VI) seemed to be the main product. In addition, products which apparently were aldonic acid esters or lactones, were also formed. Acid hydrolysis of the product mixture yielded as the only detectable aldose a compound which was indistinguishable from D-glyceraldehyde (VII) by chromatography and electrophoresis.

Oxidation of II in methanol gave mainly 2,3-*O*-isopropylidene-D-lyxose (VIII), which was further oxidized in benzene with silver carbonate



on Celite to 2,3-*O*-isopropylidene-D-lyxono-1,4-lactone (IX).

Treatment of III with the oxidant in methanol at reflux temperature resulted in formation of a mono-*O*-isopropylidene derivative of D-threose, presumably the 2,4-*O*-isopropylidene derivative (X). Hydrolysis yielded D-threose (XI), characterized as its 1,2-*O*-isopropylidene derivative (XII). When the oxidation of III was carried out in benzene-dimethylformamide, an *O*-formyl-*O*-isopropylidene-D-lyxose was the only detectable product. Removal of the *O*-formyl group yielded a compound which apparently is 3,5,-*O*-isopropylidene-D-lyxofuranose (XIII), since oxidation of this compound with silver carbonate on Celite in benzene resulted in formation of an *O*-isopropylidene-D-lyxono-1,4-lactone, different from the 2,3-*O*-isopropylidene derivative (IX). This leaves 3,5-*O*-isopropylidene-D-lyxono-1,4-lactone (XIV) as the only realistic remaining possibility. XIV was isomerized to IX in acetone in the presence of acid.

The products obtained from I, II, and III on oxidation with silver carbonate on Celite establish their structures, as far as isomerization of the *O*-isopropylidene-D-galactoses may be excluded under the reaction conditions employed. The almost neutral or weakly basic conditions make isomerizations very unlikely, and it has not been observed in oxidations of other *O*-isopropylidene aldoses.[3] The somewhat surprising formation of a lyxose derivative (XIII) from III on oxidation in benzene and dimethylformamide, whereas oxidation in methanol yields a threose derivative, is explained by the fact that the *O*-formyl group at C-4, resulting from cleavage of III between C-1 and C-2, is stable in benzene-dimethylformamide. This prevents cyclization to a new hemiacetal, and hence also prevents further degradation or oxidation to an aldonolactone. One step degradations to lyxose derivatives have also been observed when 4,6-*O*-ethylidene- and -benzylidene derivatives of D-galactose are similarly treated with this oxidant, whereas the corresponding D-glucose derivatives give negligible amounts of *O*-alkylidene derivatives of D-arabinose.[5] Oxidation of III in boiling methanol leads to a threose derivative since the *O*-formyl group initially formed undergoes transesterifications, the hydroxyl group at C-4 in the lyxose derivative then forms a hemiacetal with the aldehyde group, and further degradation may occur. In methanol the possibility also exists that degradation occurs *via* an intermolecularly formed hemiacetal, involving a molecule of methanol, and despite earlier results have shown that formation of methyl esters is more dominating than degradation of methyl acetals of some α-hydroxy aldehydes,[1,6] this seems to be the only way by which 5,6-*O*-isopropylidene-D-galactofuranose (I) can be degraded beyond the first stage.

The usefulness of anhydrous cupric sulfate and acetone in preparation of reducing mono-*O*-isopropylidene derivatives of some aldoses has previously been observed.[7,8] In particular in the presence of dimethylformamide, the formation of *O*-isopropylidene groups involving the hemiacetal hydroxyl group was inhibited.[7] The results presented in this paper support this observation. The relatively high yield of 5,6-*O*-isopropylidene-D-galactofuranose (I) should also be noticed. Except for a shorter reaction time and a relatively greater amount of dimethylformamide, the conditions are not very different from those employed in a previously reported work concerning the preparation of 1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose.[4] The 5,6-*O*-isopropylidene derivative (I) is presumably the precursor in the formation of this compound. High temperature and dimethylformamide seem to have a favorable effect on the formation of furanose forms in this reaction.

EXPERIMENTAL

Thin layer chromatography (TLC) was performed on Silica Gel G in (A) benzene-ethanol 3:1 (v/v), (B) benzene-ethanol 5:1, and (C) chloroform-methanol 25:1. Paper chromatography was done with (D) butanol-pyridine-water 5:3:1 and electrophoresis with borate buffer pH 10 on Whatman No. 1 paper. As spray reagents were used diphenylamine-aniline-phosphoric acid,[9] aniline oxalate, and for esters and lactones hydroxylamine-ferric chloride.[10] Gas liquid chromatography (GLC) was performed with a Perkin-Elmer F 11 gas chromatograph, equipped with a flame ionization detector; the column was a stainless steel column, 2 m × 2.2 mm (i.d.) with 3 % OV 225 on Gas Chrom Q 80 – 100 mesh. The temperature was maintained at 150 °C for 17 min, and then increased 4 °C/min. Mass spectra were recorded with an AEI MS 902 mass spectrometer at an ionizing potential of 70 eV.

Reaction of D-*galactose with anhydrous cupric sulfate and acetone in the presence of dimethylformamide.* D-Galactose (1 g) and anhydrous cupric sulfate (3 g) were stirred in acetone (150 ml) and dimethylformamide (30 ml) at reflux temperature for 20 h. TLC (solvents A and B) indicated the presence of five products in addition to small amounts of unreacted D-galactose. The two fastest moving compounds were indistinguishable from 1,2:3,4- and 1,2:5,6-di-*O*-isopropylidene-D-galactose, the three other products I, II, and III had R_F (TLC, solvent A) 0.46, 0.40, and 0.31, respectively. The solution was filtered, benzene (150 ml) was added and the resulting solution stirred with solid sodium hydrogencarbonate (1 g) for 30 min. After filtration and evaporation of the solvents under reduced pressure, the residue was chromatographed on a silica gel G column (30 × 3 cm) in solvent A. A fraction containing the di-*O*-isopropylidene derivatives was eluted first, and then three fractions (1, 2, and 3) were obtained, containing pure I, II, and III, respectively. A fraction containing a mixture of I and II was also obtained.

Fraction 1 gave on evaporation of the solvent and treatment of the residue with ethyl acetate, crystalline 5,6-*O*-isopropylidene-D-galactofuranose (I, 267 mg, 22 %), m.p. 83 – 84 °C, $[\alpha]_D^{22}$ – 23° (*c* 2, methanol, 5 min). (Found: C 48.86; H 7.02. Calc. for $C_9H_{16}O_6$: C 49.09; H 7.27).

Fraction 2 contained 3,4-*O*-isopropylidene-D-galactopyranose (II, 187 mg, 15 %), m.p. 100 – 102 °C after crystallization from ethyl acetate, $[\alpha]_D^{22}$ +86° (*c* 0.5, methanol, 5 min). (Found: C 49.08; H 7.18. Calc. for $C_9H_{16}O_6$: C 49.09; H 7.27).

Fraction 3 on treatment with ethyl acetate gave crystalline 4,6-*O*-isopropylidene-D-galactopyranose (III, 163 mg, 13 %), m.p. 141 – 143 °C, $[\alpha]_D^{22}$ +153° (*c* 0.5, methanol, 5 min). (Found: C 48.81; H 7.40. Calc. for $C_9H_{16}O_6$: C 49.09; H 7.27). Mass spectrum: *m/e* 205 (4, $M^+ - CH_3$), 145 (2), 131 (8), 127 (1.5) 103 (3), 101 (3), 73 (14), 59 (65), 43 (100).

All the *O*-isopropylidene derivatives (I, II, and III) gave after treatment with acetone containing 1 % (volume) sulfuric acid a compound indistinguishable from 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose by TLC (solvent B) and GLC.

*Oxidation of 5,6-*O*-isopropylidene-*D*-galactofuranose (I) in benzene.* 5,6-*O*-Isopropylidene-D-galactofuranose (I, 20 mg) was stirred in benzene (25 ml) at reflux temperature for 15 min, silver carbonate on Celite (1 g) was then added, and the stirring under reflux continued for 20 min. TLC (solvent A) showed a single product, detectable with the ester reagent only, and indistinguishable from 5,6-*O*-isopropylidene-D-galactono-1,4-lactone (IV), prepared as described below. Filtration of the solution and evaporation of the solvent gave a syrupy residue (15 mg), $[\alpha]_D^{22}$ –45° (*c* 1, acetone). The infrared spectrum was identical with that of the authentic sample (strong absorption at 1780 cm^{-1} in $CHCl_3$).

*5,6-*O*-Isopropylidene-*D*-galactono-1,4-lactone (IV) from* D-*galactono-1,4-lactone (V).* D-Galactono-1,4-lactone (V, 0.5 g) was stirred with anhydrous cupric sulfate (3 g) in acetone (100 ml) for 18 h. TLC (solvent A) showed the presence of traces of starting material, and a single product with higher mobility. The solution was filtered and the solvent evaporated. Extraction of the residue with a hot mixture of benzene and ethyl acetate 3:2 afforded after removal of the solvents a chromatographically homogeneous syrup, $[\alpha]_D^{22}$ –42° (*c* 2, acetone), the infrared spectrum ($CHCl_3$) showed strong absorption at 1780 cm^{-1}, characteristic of 1,4-lactones. (Found: C 48.42; H 6.45. Calc. for $C_9H_{14}O_6$: C 49.54; H 6.42).

Treatment of the syrup with sodium periodate in water at pH 7 (adjusted by addition of sodium hydrogencarbonate), extraction of the water solution with chloroform and evaporation of the chloroform, gave a product indistinguishable (TLC, solvents B and C) from 2,3-*O*-isopropylidene-D-glyceraldehyde (VI). Hydrolysis of this compound in 30 % aqueous acetic acid at 40 °C for 2 h, yielded as the only detectable product a compound indistinguishable from D-glyceraldehyde (VII) by TLC (solvent A) and electrophoresis (M_{glc} 0.73).

*Oxidation of 5,6-*O*-isopropylidene-*D*-galactofuranose (I) in methanol.* 5,6-*O*-Isopropylidene-D-galactofuranose (I, 30 mg) in methanol (5 ml) was stirred with silver carbonate on Celite at reflux temperature for 90 min. TLC (solvent B) showed minor amounts of two compounds detectable with hydroxylamine-ferric chloride, R_F 0.54 and 0.40, and one component detectable with diphenylamine-aniline-phosphoric acid, R_F 0.48, indistinguishable from 2,3-*O*-isopropylidene-D-glyceraldehyde in this solvent and in solvent C.

Preparative chromatography of this compound (TLC, solvent B) after filtration of the solution and evaporation of the solvent gave a chromatographically pure 2,3-*O*-isopropylidene-D-glyceraldehyde (VI), $[\alpha]_D^{22}$ +60° (*c* 1, benzene) (lit.[11] –67.9° for the L-enantiomer).

Hydrolysis in 30 % aqueous acetic acid at 40 °C for 2 h, evaporation of the water and acetic acid and treatment of the residue in water with Dowex 1 (HCO_3^-) ion exchanger yielded after evaporation of the solvent a syrup. TLC (solvent A) and electrophoresis showed a single compound, detectable with diphenylamine-aniline-phosphoric acid, indistinguishable from D-glyceraldehyde (VII).

*Oxidation of 3,4-*O*-isopropylidene-*D*-galactopyranose (II).* 3,4-*O*-isopropylidene-D-galactopyranose (II, 30 mg) in methanol (5 ml) was stirred under reflux with silver carbonate on Celite (0.5 g) for 15 min. The solution was filtered and the solvent evaporated yielding a syrup; TLC (solvent B) showed the presence of a major compound, detectable with diphenylamine-aniline-phosphoric acid, indistinguishable

from 2,3-*O*-isopropylidene-D-lyxose (VIII). Minor amounts of two compounds detectable with the ester reagent, were also present. GLC also showed the presence of a major compound, retention time 10.5 min, indistinguishable from VIII.

Oxidation of the product mixture with silver carbonate on Celite (0.5 g) in benzene (10 ml) at reflux temperature for 1 h, filtration of the solution and evaporation of the solvent, gave a syrup from which crude 2,3-*O*-isopropylidene-D-lyxono-1,4-lactone (IX) crystallized on treatment with ethyl acetate-light petroleum (b.p. 60 – 80 °C). The yield was 15 mg, m.p. 88 – 93 °C (lit.[12] 99 – 100 °C), $[\alpha]_D^{22}$ +108° (*c* 1, acetone) (lit.[12] +106°, water). The product was indistinguishable from an authentic sample of IX by GLC (retention time 25.2 min) and TLC (solvents B and C); the infrared spectra were identical ($CHCl_3$, strong absorption at 1785 cm^{-1}).

*Oxidation of 4,6-*O*-isopropylidene-*D*-galactopyranose (III) in methanol.* 4,6-*O*-Isopropylidene-D-galactopyranose (III, 75 mg) in methanol (25 ml) was stirred at reflux temperature for 30 min with silver carbonate on Celite (2 g). TLC (solvent B) showed a single component detectable with diphenylamine-aniline-phosphoric acid, giving the same colour as 1,2-*O*-isopropylidene-β-D-threofuranose (XII); the mobility relative to that of XII was 0.75. In addition, small amounts of a slightly slower moving compound, detectable with the ester reagent, were also observed. The solution was filtered and the solvent evaporated to give a syrupy residue which was hydrolyzed in 30 % aqueous acetic acid for 2 h at 45 °C. After removal of the solvents, TLC (solvent A) showed only one compound detectable with diphenylamine-aniline-phosphoric acid, indistinguishable from D-threose (XI). Treatment of the product with acetone (10 ml) containing 1 % sulfuric acid for 2 h, neutralization with solid sodium hydrogencarbonate, filtration and evaporation of the solvent gave a syrup (35 mg) from which a few crystals of 1,2-*O*-isopropylidene-β-D-threofuranose were obtained on treatment with ethyl acetate-light petroleum, m.p. 76 – 81 °C (lit.[13] 83 – 84 °C). Preparative of TLC the syrup (solvent B) yielded chromatographically homogeneous 1,2-*O*-isopropylidene-β-D-threofuranose (XII), the yield was 14 mg, $[\alpha]_D^{22}$ −15° (*c* 1, acetone) (lit.[13] – 15.1°).

*Oxidation of 4,6-*O*-isopropylidene-*D*-galactopyranose (III) in benzene and dimethylformamide.* 4,6-*O*-Isopropylidene-D-galactopyranose (III, 95 mg) in dimethylformamide (20 ml) was poured into a stirred mixture of silver carbonate on Celite (2 g) and benzene (100 ml) at 80 °C. The stirring was continued for 25 min at reflux temperature, the solution was then filtered and the solvents evaporated. Treatment of the residue with Dowex 1 (HCO_3^-) ion exchanger in methanol and subsequent removal of the solvent yielded a chromatographically (TLC, solvent B) homogeneous syrup (62 mg), the mobility relative to that of 2,3-*O*-isopropylidene-D-lyxose (VIII) was 0.84. Hydrolysis of a part of the product (5 mg) in 3 % aqueous acetic acid at 45 °C for 2 h and subsequent removal of the solvents afforded a product indistinguishable from D-lyxose by electrophoresis (M_{glc} 0.67) and paper chromatography.

The remaining part (57 mg) of the above product was oxidized with silver carbonate on Celite (3 g) in benzene (50 ml) at reflux temperature for 75 min. The solution was filtered and the solvent removed giving a product which crystallized from ethyl acetate-light petroleum (40 mg). The compound, 3,5-*O*-isopropylidene-D-lyxono-1,4-lactone (XIV), had m.p. 137 – 138 °C, $[\alpha]_D^{22}$ +22° (c 2, acetone), the infrared spectrum showed strong absorption at 1785 cm^{-1} (in $CHCl_3$), characteristic of 1,4-lactones. The mobility (TLC, solvent C) relative to that of 2,3-*O*-isopropylidene-D-lyxono-1,4-lactone (IX) was 0.86. Mass spectrum: *m/e* 173 (9, $M^+ - 15$), 159 (2), 149 (1), 145 (2), 131 (7), 130 (6), 113 (4), 101 (16), 85(12), 73 (5), 59 (61), 43 (100).

The lactone XIV (30 mg) was treated with acetone (10 ml) containing 1 % sulfuric acid for 2 h. The solution was neutralized with solid sodium hydrogencarbonate and filtered. TLC showed the presence of a compound indistinguishable from 2,3-*O*-isopropylidene-D-lyxono-1,4-lactone (IX) (solvents B and C). In addition, substantial amounts of a compound with mobility corresponding to that of the starting material were also present.

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