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Reaction of D-Glucosone with Methanol-Hydrogen Chloride

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D-Glucosone (D-*arabino*-hexosulose) has been treated with methanol and hydrogen chloride under the conditions for the Fischer glycoside synthesis. The two major products were isolated. The first one, amorphous, was shown to be D-*arabino*-hexosulose 1,1-(dimethyl acetal) and the other one, crystalline, methyl β-(D-*arabino*-hexosulo-furanoside) 1,1-(dimethyl acetal).

The yields of "keto-glucosides" obtained by oxidation of methyl D-glucopyranosides, or their derivatives, are low owing to different side-reactions [1]. Osones are easily prepared in good yields from aldoses by oxidation with cupric acetate [2], and the possibility of preparing "2-keto-glucosides" by treatment of D-glucosone with methanol-hydrogen chloride (in analogy with the Fischer glucoside synthesis) was therefore investigated. Under these conditions hexoses yield equilibrium mixtures containing anomeric methyl furanosides and pyranosides. A dicarbonyl carbohydrate as D-glucosone is expected to give a much more complex equilibrium mixture. As hemiacetal ring structures, with a free (or hydrated) keto group have been considered to predominate in an equilibrated solution [3] of D-glucosone, the formation of "2 ketoglucosides" was expected.

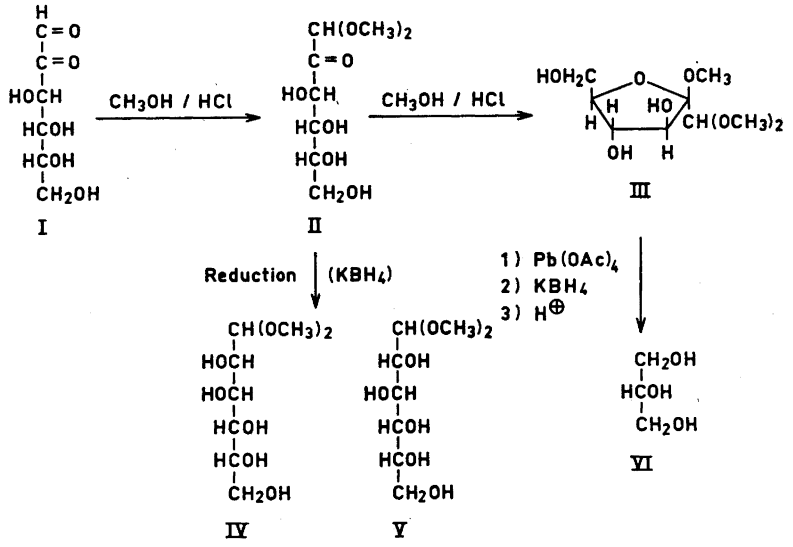
In a preliminary experiment a small amount of chromatographically pure D-glucosone (I) was treated with dry methanol containing 4 % hydrogen chloride at room temperature for one day. In the product, however, only traces of compounds were present, which behaved on paper chromatograms like the anomeric methyl "2-keto-glucopyranosides". The two predominating compounds present did not give the characteristic brick-red colour with resorcinol hydrogen chloride of the "ketoglucopyranosides" but a pink colour as D-glucosone itself. The two components were isolated by chromatography on thick papers. The one with the lower R_F-value (II) gave glucose and mannose but no fructose on borohydride reduction and subsequent hydrolysis, indicating reduction at C-2. Like ketoses but unlike D-glucosone, it did not migrate on electrophoresis in hydrogen sulphite [4]. The other compound (III) gave II and glucosone on mild hydrolysis.

Greater amounts of the two predominating components were obtained by treatment of a crude mixture of D-glucose and D-glucosone with methanolic hydrogen chloride and fractionation of the product on carbon and cellulose

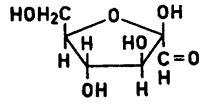
columns. The isolation of II and III was simplified by subjecting the product to a mild acid treatment, which did not significantly reduce the amounts of these substances. The D-glucofuranosides, however, were hydrolysed to D-glucose, which in contrast to the furanosides, was easily separated from II and III.

Substance II, $[\alpha]_D$ −28°, which did not crystallise, contained two methoxyl groups and was shown to be D-*arabino*-hexosulose 1,1-(dimethyl acetal), by the isolation of the crystalline dimethyl acetals (IV and V) of D-mannose and D-glucose (in the proportions 2.6:1) after borohydride reduction.

Substance III was obtained crystalline, m.p. 97—98°, $[\alpha]_D$ −20.8°, and contained three methoxyl groups. It was the main product when II was treated with methanolic hydrogen chloride, and is obviously a methyl glycoside of II. It did not migrate on electrophoresis in borate, which indicates a furanosidic structure. Compare the analogous methyl D-arabinofuranosides [M_G 0.04) and the methyl D-arabinopyranosides (M_G 0.38) [5]. The low optical rotation suggests a β-configuration. On lead tetraacetate oxidation in pyridine [6], the substance consumed one mole of oxidant in agreement with the furanosidic structure. Further, glycerol (VI) was isolated after borohydride reduction and hydrolysis of the oxidation product.



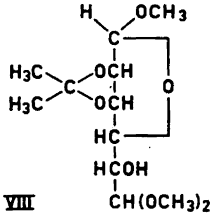
II and III were isolated in 20 and 24 % yields calculated on the original D-glucosone (however some unreacted D-glucosone was also present). As significant amounts of II and III were lost in the overlaping fractions during the chromatographic separation steps, their percentages in the mixture of reaction products must be much higher. If one can assume that the composition of the equilibrium mixture obtained in the Fischer synthesis is similar to that of the free D-glucosone in equilibrium, the present results strongly suggest that D-glucosone exists predominantly with a furanosidic (preferentially β-furanoside) hemiketal structure and contains a free (or hydrated) aldehyde group (VII).



VII

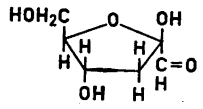
The electrophoretic mobility on hydrogen sulphite electrophoresis [4] of D-glucosone (M_V 1.24) compared with the high mobility of *aldehydo*-D-glucose-pentaacetate (M_V 1.18) and the low mobilities of D-glucose (M_V 0.05) and D-fructose (M_V 0.00) lends further support to this suggestion. The structure is also in agreement with the known partial reduction of D-glucosone to D-fructose [7].

The direct preparation of dialkyl acetals of tetroses and higher sugars has not been reported, but Ballou and Fischer [8] obtained an amorphous compound to which was assigned the structure VIII when 2,3:4,5-di-*O*-isopropylidene-D-*manno*-hexodialdose was treated with methanolic hydrogen chloride. In VIII only one of the aldehyde groups can form a hemiacetal ring. Dithioacetals, however, are formed directly by reaction between sugars and thiols in acidic solution. Bayne [9] has reported the preparation of crystalline osone 1,1-(diethyl dithioacetals) by this route.

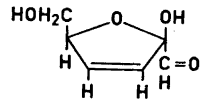


VIII

Wolfrom and coworkers [10] have postulated and Anet [11] has recently shown that 3-deoxy-D-*erythro*-hexosulose and 3,4-dideoxy-D-*glycero*-hexosulose-3-ene are intermediates in the acidic degradation of hexoses to 5-(hydroxymethyl)-2-furaldehyde. In the light of the present investigation one can assume that these hexosuloses also exist to a great extent as the hemiketals, (IX) and (X), with a free or hydrated aldehyde group. As they already contain the required ring structure, their ease of transformation into 5-(hydroxymethyl)-2-furaldehyde is then more readily understood.



IX



X

EXPERIMENTAL

All melting points are corrected. Concentrations were carried out under reduced pressure below 40°.

Paper chromatography and electrophoresis

Whatman No. 1 and 3 MM (water washed, for preparative separations) filter papers were used.

Solvents and electrolytes:

A. Butan-1-ol-ethanol-water, 10:3:5.
B. Ethyl acetate-acetic acid-water, 3:1:1.
C. Methyl ethyl ketone, saturated with water.
D. Hydrogen sulphite pH 4.7, 0.1 M (used at 50°).
E. Borate pH 10.0, 0.1 M.

Spraying reagents:

a. Silver nitrate-sodium hydroxide.
b. Anisidine hydrogen chloride.
c. Resorcinol-hydrochloric acid.

Treatment of D-glucosone with methanol/hydrogen chloride

Preliminary experiments. In preliminary experiments pure D-glucosone, prepared by oxidation of D-glucose with cupric acetate [2] and purified by chromatography on a carbon column was used. A sample (0.20 g) was treated with dry methanolic hydrogen chloride (10 ml, 4 % HCl) at room temperature. Small samples were withdrawn at intervals, deionised and examined by paper chromatography. After 24 h the chromatographic picture of the main components was essentially unchanged and the treatment was interrupted after 30 h and the solution was deionised. In the complex mixture some D-glucosone still remained and two components were clearly preponderant. They appeared as pink spots, similar to D-glucosone, with reagent c and had the R_L-values 0.38 (II) and 0.59 (III), respectively, in solvent A. Using this solvent the two components were isolated by chromatography on thick papers. A paper chromatographic examination of the hydrolysate after mild acid treatment of III revealed that it yielded II and glucosone. After borohydride reduction and hydrolysis of II, glucose and mannose but no fructose was detected.

Amongst the many other minor components traces of compounds with similar R_F-values as those of the two anomeric "2-keto-glucopyranosides" and giving the characteristic brick-red colour with reagent c, were detected.

Larger-scale experiment. In the larger-scale experiment the rather time-consuming separation of D-glucose and D-glucosone after the oxidation was not made.

D-Glucose (40.00 g) in water (80 ml) and methanol (1900 ml) was oxidised by cupric acetate (165.00 g) by quickly raising the temperature to boiling and then refluxing for 10 min. The mixture was cooled, filtered to remove cuprous oxide and the copper in the filtrate was removed by hydrogen sulphide. The filtrate was treated with some charcoal before the final concentration to a colourless syrup. The water in this was removed by repeated addition and distillation of ethanol and benzene resulting in a syrup (35.92 g). A semi-quantitative determination by paper electrophoresis in electrolyte D and visual comparison of spot areas, using solutions of known concentration as reference showed a D-glucosone content of 14.5 g in the syrup.

The product was dissolved in dry methanolic hydrogen chloride (800 ml, 4 % HCl) and kept at 22° for 25 h. Water (270 ml) was added and the mixture was quickly brought to boiling and kept refluxing for 10 min. After cooling and deionisation with an anion exchange resin (Dowex 3, free base) the product was evaporated to a syrup (33.80 g).

Chromatographic resolution of the reaction products

The product (above) consisted of a complex mixture with D-glucose, methyl D-glucopyranosides and II and III as major components. There were also small amounts of several other components, including glucosone, none of which constituted more than *ca.* 20 % of either of the components II and III.

A crude fractionation was first made on a carbon-Celite column (71 × 8 cm) which was eluted as follows: water (5 l), gradient elution with aqueous ethanol (10 l, 0—5 % and 15 l, 5—20 %) and 50 % ethanol (10 l). After a paper chromatographic examination the subfractions were combined to four main fractions, containing as major components (*a*) D-glucose and D-glucosone, (*b*) methyl D-glucopyranosides, (*c*) methyl D-glucopyranosides + II and (*d*) III, respectively.

Fraction (*c*) (10.62 g) was further fractionated on a carbon-Celite column (52 × 6.5 cm) using aqueous ethanol (12 l, 0—12 %) and the main fraction, enriched in II, fractionated again in the same way giving a main fraction of 5.22 g. Half of this was resolved by chromatography on a cellulose column (55 × 5 cm) using butan-1-ol, saturated with water as solvent. A chromatographically pure, syrupy fraction (1.83 g, when dried in vacuum over P_2O_5) of II was obtained. This corresponds to a total yield of 20 % of the original D-glucosone.

After two fractionations on a carbon-Celite column (29 × 6 cm) using aqueous ethanol (6 l, 0—10 % and 5 l, 10—20 %) fraction (d) (8.92 g) yielded a main fraction of 5.89 g. Half of this was refractionated on the same column (3 l, 0—10 % and 10 l, 10—20 %) from which a syrupy fraction (2.44 g, when dried in vacuum over P_2O_5) of III with only traces of impurities was obtained. This corresponds to a total yield of 24 % of the original D-glucosone. After addition of ethanol the syrup slowly crystallised. Recrystallisation from ethanol-isopropyl ether gave 1.41 g of pure III. Two of the components in fraction (*d*) were isolated by separating an aliquot on thick papers. They both gave glucosone by hydrolysis, but only one of them, giving pink colour with reagent c, yielded II as intermediate. The other one gave a yellowish colour with reagent c.

D-Arabino-*hexosulose 1,1-(dimethyl acetal) (II)*. The optical rotation of the amorphous compound was $[\alpha]_D^{22}$ −28° (water, *c* 2.0) and the methoxyl content 25.5 % (calc. for $C_8H_{16}O_7$; OCH_3 27.7 %). It gave a strong reaction with reagent a, shortly after spraying, and a pink colour with reagent c. R_G-values were 1.65, 1.92 and 3.6 (solvent A, B, and C, respectively) and the M_V-value 0 (electrolyte D).

A sample of II (170 mg) was reduced with an excess of potassium borohydride in aqueous solution of pH 10. After deionisation and separation on thick papers (2 × 18 h) using solvent C two fractions were obtained. After evaporating the eluates, dissolving in ethanol, filtering and evaporation, the two fractions amounted to 92 and 36 mg, respectively. The first one, indistinguishable from D-mannose dimethyl acetal (IV) (R_G 3.36 and M_G 0.69 in C and E, respectively) was recrystallised from methanol-ethyl acetate, m.p. 98—99° (m.p. and mixed m.p. identical with an authentic sample). The second one, indistinguishable from D-glucose dimethyl acetal (V) (R_G 4.10 and M_G 0.74 in C and E, respectively) was also recrystallised from methanol-ethyl acetate, m.p. 94—95° (m.p. and mixed m.p. identical with an authentic sample).

Another sample of II (100 mg) was treated with dry methanolic hydrogen chloride (2 ml, 4 % HCl) at room temperature for 25 h. From the deionised product, which consisted of III (major constituent), 20—30 % II and some minor components, III (35 mg) was crystallised directly. After recrystallisation from ethanol-isopropylether it melted at 97—98° (m.p. and mixed m.p. identical with that of III).

*Methyl β-(*D-arabino-*hexosulo-furanoside)1,1-(dimethyl acetal) (III)*. After recrystallisation from ethanol-isopropyl ether, m.p. 97—98°, $[\alpha]_D^{22}$ −20.8 (water, *c* 2.0), (Found: C 45.5; H 7.40; O 47.0; OCH_3 39.3. $C_9H_{18}O_7$ requires C 45.5; H 7.57; O 47.1; OCH_3 39.1). The substance gave a weak greyish spot with reagent a, some time after the spraying, and a pink colour with reagent c. The R_G-values were 2.62, 2.77 and 11.2 (in solvent A, B, and C, respectively) and M_G 0 (in electrolyte E).

The substance reacted very slowly with metaperiodate. With lead tetraacetate in pyridine [6] it reacted readily consuming 1 mole of oxidant/mole III; thus 14.1 mg III treated with lead tetraacetate (120 mg) in pyridine (1 ml) at 0° reacted as follows:

Time (min.):	5	15	35	65	120
Moles of oxidant/mole III:	0.80	0.91	1.00	1.09	1.13

Another sample (100 mg) was treated with lead tetraacetate (940 mg) in pyridine (8 ml) and kept for 45 min. at 0°. To the reaction mixture was then added a few drops of water and oxalic acid (2.00 g), the precipitate was filtered off, the filtrate concentrated

and the residue obtained was taken up in warm acetone and filtered. After evaporation, the product was reduced with excess potassium borohydride in aqueous solution at pH 10. After deionisation and concentration (90 mg syrup) the product was hydrolysed in 0.5 N sulphuric acid (5 ml) at 96° for 1 h. After neutralisation with barium carbonate, filtration and evaporation the product was separated on thick filter papers (solvent A) and a fraction (29 mg) indistinguishable from glycerol with small amounts of impurities was collected. There were no traces of ethylene glycol present. The glycerol fraction was benzoylated by the Schotten-Bauman procedure, yielding a crystalline tri-benzoate (32 mg) which after recrystallisation from aqueous ethanol had m.p. 75—76° (m.p. and mixed m.p. identical with an authentic sample).

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Degradation Products of γ-Irradiated Solid Carbohydrates Obtained after Dissolution in Water

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It is shown by paper chromatographic analysis that a number of substances are formed when γ-irradiated solid carbohydrates are dissolved in water. CO_2 is formed from α-D-glucose with a G-value of 2.5, while D-fructose and sucrose gave no CO_2. The production of acidic groups of different carbohydrates showed a wide range of G-values, *e.g.* 1.0 for sucrose and 48 for lactose · H_2O.

We have earlier studied radiation damage of solid carbohydrates by means of electron spin resonance [1,2], and the reactions occurring after the irradiated material is dissolved in water [1,3,4]. Alongside with this work, efforts have been made to separate and identify the degradation products. Apart from the radiation chemistry viewpoint, it seemed to be of interest to isolate and identify these degradation products as some of them might have reactive properties causing indirect chemical and biological effects [3,5–7]. The present communication will give preliminary results of this work, mainly on α-D-glucose.

The radiation damage of carbohydrates in aqueous solution has been extensively studied [8–13]. Only a few data have been reported on degradation products obtained after dissolution of irradiated solid carbohydrates. Wolfrom *et al.*[12,13], using paper chromatography, found that sucrose gives glucose and fructose and that methyl-α-D-glucoside gives glucose. Collins[14] also using paper chromatographic analysis states that only glucose is present in the endproduct after irradiation of glucose. Grant and Ward[11] refer to an unpublished work by Bourne, Hudson and Weigel, stating that auto-radiolysis of ^{14}C-labelled glucose gives a mixture similar to that obtained in dilute aqueous solution *in vacuo*. According to the work of Grant and Ward these products are gluconic acid, 2-oxo-D-*arabino*-aldohexose, 2-oxo-D-*arabino*-hexonic acid, arabinose, arabonic acid and glucosaccharic acid.