Glucosan, prepared in the manner of xylosan, has properties which closely resemble xylosan.

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THE MECHANISM OF CARBOHYDRATE OXIDATION. XV.[1] THE BEHAVIOR OF 3-GLUCOSIDO-ARABINOSE HEPTA-ACETATE TOWARD AQUEOUS SOLUTIONS OF POTASSIUM HYDROXIDE

BY WM. LLOYD EVANS AND CHARLES C. CLARK

It has been shown recently that there is a marked difference in the behavior of the 4-hexosido-glucoses and the 6-hexosido-glucoses toward aqueous solutions of potassium hydroxide.[1] These differences were attributed principally to the following general causes: (a) the chemical character of the hexosido section of the disaccharide molecule; and (b) the point of the hexosidic attachment. When maltose and cellobiose are acted upon by aqueous solutions of potassium hydroxide, it is found that the yields of lactic acid from these two disaccharides are about one-half as much as one would expect from an equivalent solution of glucose. In seeking an explanation for this latter fact through the application of the enediol theory of chemical behavior in the carbohydrates, it was shown that maltose and cellobiose should form a 3-glucosido-arabinose and formaldehyde as their first decomposition products in each case. Therefore, it becomes evident that these intermediate 3-glucosido-arabinoses obtained from maltose and cellobiose should be expected to show the same general reaction toward aqueous solutions of potassium hydroxide as did the original disaccharides under the same experimental conditions. If this assumption is correct, then in the alkaline decomposition of the 3-glucosido-arabinoses one should obtain pyruvic aldehyde, lactic and formic acids in yields of approximately the same order of magnitude as those obtained from cellobiose and maltose. To establish these points experimentally was the principal objective in this work.

Experimental Part

Cellobiose Octa-acetate.—The method used for the preparation of cellobiose octa-acetate was essentially that described by Zemplén.[2] If the heat of reaction was insufficient to cause the temperature to rise to 100°, the mixture was heated on an electric hot-plate to that point. Our experience coincided with that of Haworth and Hirst,[3,4] who found that it was better to judge the progress of the reaction by the color of the

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[1] Contribution XIV of this series, THIS JOURNAL, 53, 4384 (1931).

[2] Zemplén, Ber., 59, 1254 (1926).

[3] Haworth and Hirst, J. Chem. Soc., 2809 (1927).

[4] Cf. Haworth, Hirst, Streight, Thomas and Well, ibid., 2636 (1930).

reaction mixture rather than by its temperature alone; *i. e.*, when the color is changing rapidly from a light to a dark red the reaction should be stopped. The yellowish-brown gummy precipitate that separated upon pouring the reaction mixture into water was allowed to stand overnight, after which it was filtered by suction, washed twice with water and then recrystallized once from twice its weight of alcohol–acetic acid mixture (5:1), according to the method of Levene and Wolfrom.[5] The average yield was 12 g. (m. p. 221°) from 60 g. of filter paper.

Cellobiose.—Cellobiose was prepared from the octa-acetate by the method of Zemplén.[2] The yields obtained in this procedure varied from 80–90%.

Preparation of 3-Glucosido-arabinose Hepta-acetate.—The procedure used was that of Zemplén.[2] A modification introduced by Levene and Wolfrom[5] in the preparation of cellobiose oxime was employed, *viz.*, calcium carbonate was used in evaporating the reaction mixture *in vacuo* to avoid the presence of free acid. The oxime was crystallized by nucleation.

Treatment of 3-Glucosido-arabinose Hepta-acetate with Potassium Hydroxide.—Eight-tenths gram (0.25 M) samples of crystalline 3-glucosido-arabinose hepta-acetate (m. p 193°) were agitated with 5.27 cc. of potassium hydroxide solutions of the appropriate normalities at 25 and 50° for forty-eight hours. The procedure adopted was the same as that previously employed in this Laboratory for work of the same type,[6] with the exception that this is the first time that the acetate of a sugar has been employed in an investigation of this nature. Its use was necessitated because the non-acetylated carbohydrate could not be crystallized. Consequently, the alkaline solutions had to be of sufficient normality to deacetylate the acetate and also to react with the free sugar. In the experiments with mixtures of sugar acetates, 0.515 g. of glucose penta-acetate and 0.42 g. of arabinose tetra-acetate were used.

Formic and Acetic Acids.—Formic acid was determined by the method previously described.[6] The amount of acetic acid formed from the enolic splitting of 3-glucosido-arabinose was too small to be determined accurately by difference in the presence of the large amount of this same acid produced by the saponification of the acetate. For this reason no attempt was made to secure data for acetic acid.

Pyruvic Aldehyde Osazone.—The osazone experiments were carried out in the same manner as described in previous papers[6] except that 0.8-g. samples of the sugar derivative were employed because of the scarcity of the material. The volume of standard potassium hydroxide solution was 5.27 cc., that of phenylhydrazine 1.09 cc., and alcohol (95%) 3.70 cc. In determining the normality of the potassium hydroxide solutions, corrections were applied for the volume of the alcohol and the phenylhydrazine.

Lactic Acid.—This acid was determined by a method developed by G. F. Nadeau[7] of this Laboratory. The details which are to be published later are essentially as follows. Barium carbonate is used instead of zinc carbonate as the compound over which the ether extract of lactic acid is collected. The residue remaining after the evaporation of the ether was treated with water and the insoluble barium carbonate removed. The filtrate containing the soluble barium lactate was evaporated to dryness on a steam-bath. The barium lactate was removed from the salt mixture by means of 80% alcohol and the resulting solution was analyzed for its barium content.

Experimental Data.—Our experimental data are given in graphical form in Figs. 1–5.

[5] Levene and Wolfrom, *J. Biol. Chem.*, **77**, 677 (1928).

[6] Evans and Benoy, THIS JOURNAL, **52**, 294 (1930).

[7] G. F. Nadeau, Dissertation for the Ph.D. degree, The Ohio State University, March, 1931.

Discussion and Summary

Theory.—It has been pointed out by Benoy[6] and Hockett[1] and one of us that the 3-glucosido-arabinoses obtained in the decomposition of the 1,2-enediols of maltose and cellobiose should undergo in turn the same kind of changes in aqueous solutions of potassium hydroxide as the disaccharides themselves do. In these substituted pentoses, as in maltose and cellobiose, there is still present the hindering action[8,1] which arises from the point of glucosidic attachment (carbon atom 3), which necessarily imposes a limit on the number of enediols which may possibly form under these circumstances. It is conceivable that these 3-glucosido-arabinoses may undergo decomposition reactions in three possible ways: (a) the pentose derivative may hydrolyze, thus giving rise to glucose and arabinose; or (b) there is no *a priori* reason why the 3-glucosido-arabinoses should not be converted to the corresponding 3-glucosido-arabinuloses and 3-glucosido-riboses through the well-known de Bruyn and van Ekenstein rearrangement,[9] a reaction which would be followed by the formation of a common 3-glucosido-pentose-1,2-enediol; and (c) both reactions (a and b) may take place simultaneously. A comparison of the relative amounts of pyruvic aldehyde, lactic and formic acids obtained from alkaline solution of 3-glucosido-arabinose hepta-acetate from cellobiose with the quantities of the same products obtained from maltose, cellobiose, glucose and a mixture of glucose penta-acetate and arabinose tetra-acetate, is best understood on the general basis outlined in this paragraph, and also from the following more detailed statement of the mechanism.

Lactic Acid.—If 3-glucosido-arabinose hepta-acetate (from cellobiose) undergoes a rapid hydrolysis in aqueous solutions of potassium hydroxide such as one would expect in acid solutions, then both the hexose and pentose sugars thus formed would become sources[10] for the production of lactic acid. Under these circumstances the amount of this acid should more nearly approximate that obtained from an equivalent mixture of glucose pentaacetate and arabinose tetracetate[11] as shown in Fig. 1. However, a comparison of the data obtained from the reaction mixture of these two acetates with those obtained from the 3-glucosido-arabinose hepta-acetate shows that the latter sugar derivative yields but approximately half of the lactic acid one should expect if the 3-glucosido-arabinose hepta-acetate had first undergone complete hydrolysis. Obviously, one must conclude,

[8] Lewis and Buckborough, This Journal, **36**, 2385 (1914); Nef, *Ann.*, **403**, 204 (1914).

[9] De Bruyn and van Ekenstein, *Rec. trav. chim.*, **14**, 156, 203 (1895); **15**, 92 (1896); **16**, 257, 262, 274 (1897); **19**, 1 (1900); **27**, 1 (1908). *Cf.* Gross with Lewis, This Journal, **53**, 2772 (1931).

[10] Evans and O'Donnell, *ibid.*, **50**, 2543 (1928); Evans and Conaway, *ibid.*, **52**, 3680 (1930).

[11] Hudson and Dale, *ibid.*, **40**, 994 (1918).

therefore, that the reaction mechanism for the production of lactic acid through the preliminary hydrolysis of 3-glucosido-arabinose is not wholly adequate and, indeed, accounts for but a small fraction of this product. The other alternative lies in the further decomposition of 3-glucosido-arabinose into 2-glucosido-erythrose and hydroxymethylene and the
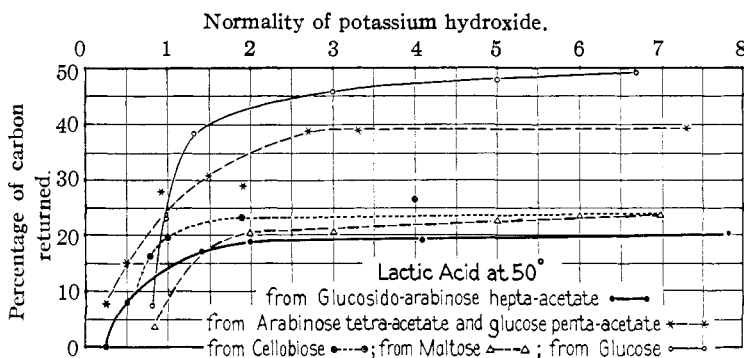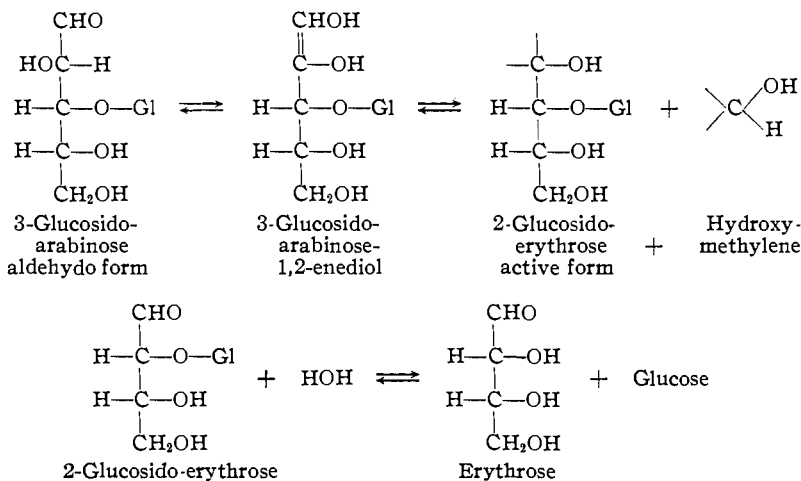

Fig. 1.

subsequent hydrolysis of the tetrose derivative as shown by the following reactions in which it is assumed that a complete deacetylation had taken place previously.

These reactions show that it is theoretically possible for glucose to be formed as a reaction product through a series of steps that would be equivalent to changing the pentose section of the reacting molecule into one of a tetrose sugar and one of hydroxymethylene. This, in effect, would remove the possibility of the arabinose being a source of lactic acid because

it has been shown by Hutchman[12] of this Laboratory, that no lactic acid is formed in alkaline solutions of glycol aldehyde, solutions which Fischer and Landsteiner[13] have shown certain tetrose sugars. From these facts it follows that the lactic acid produced in the action of aqueous solutions of potassium hydroxide on 3-glucosido-arabinose hepta-acetate must be derived from the hexosido section of the molecule. This conclusion is in complete harmony with the following experimental facts. (a) Lactic acid is formed in aqueous solutions of the hexoses in the presence of potassium hydroxide and in amounts that are dependent upon the original alkali normality of the solutions and the temperature employed, factors[10] which also seem to play an important role in the alkaline decomposition of 3-glucosido-arabinose. (b) Lactic acid is also found in the alkaline solutions of cellobiose and maltose (Fig. 1) in amounts that harmonize with the postulate that the hexosido section of these molecules is the source of this
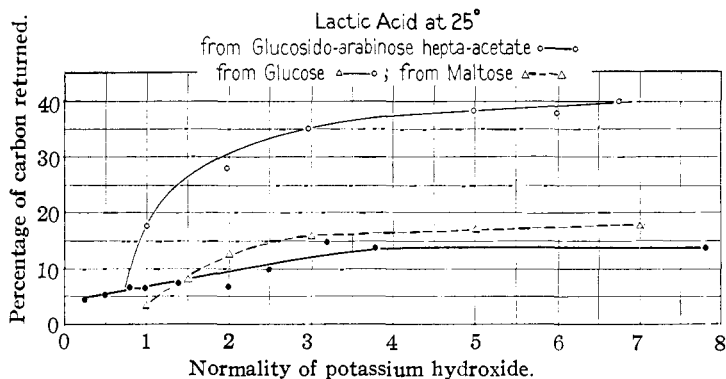

Fig. 2.

product, i. e., the amounts of lactic acid obtained from these two disaccharides were found to be approximately one-half those derived from an equivalent solution of glucose under the same experimental conditions. The yields of lactic acid in these latter cases are also dependent upon the experimental factors given in (a). The effect of temperature on the production of lactic acid from alkaline solutions of maltose, glucose and 3-glucosido-arabinose hepta-acetate may be seen by comparing the data given in Figs. 1 and 2.

From these considerations it is seen that the behavior of 3-glucosido-arabinose toward aqueous solutions of potassium hydroxide harmonizes with the postulate that this pentose derivative is an intermediate compound in the alkaline degradation of cellobiose and maltose; i. e., as is shown by

[12] J. E. Hutchman, Dissertation for the Ph.D. degree, The Ohio State University, 1927; cf. Ref. 1.

[13] Fischer and Landsteiner, Ber., 25, 2549 (1892).

our experimental data in Fig. 1, the amount of lactic acid obtained from the alkaline degradation of 3-glucosido-arabinose hepta-acetate is approximately the same as that derived from these two disaccharides under the same experimental conditions.
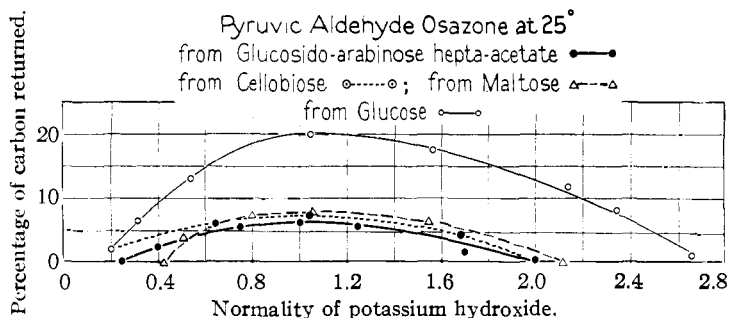

Fig. 3.

Pyruvic Aldehyde.—Since pyruvic aldehyde is regarded as the forerunner of lactic acid formation in the action of alkalies on the carbohydrates, it was to have been expected that the amounts of pyruvic aldehyde osazone formed from 3-glucosido-arabinose hepta-acetate should
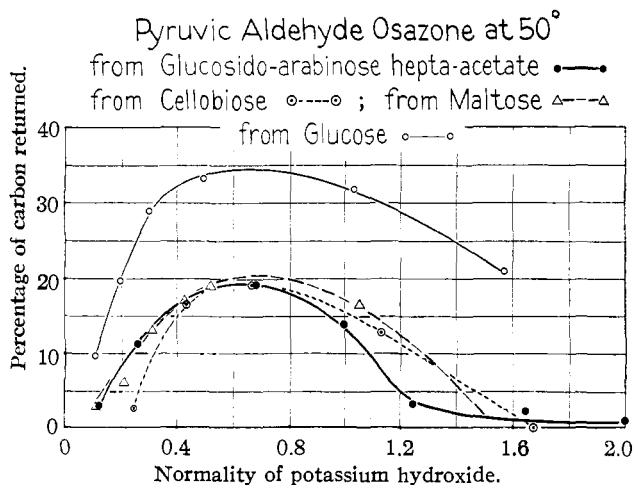

Fig. 4.

show the same general relationship to the yields of this derivative obtained from glucose, cellobiose and maltose as in the case with the yields of lactic acid. That this seems to be true at 25 and 50° is shown in Figs. 3 and 4, respectively.

Formic Acid.—The sources of formic acid in the alkaline degradation of the disaccharide sugars have been pointed out previously.[1,6,10]   It was

shown that the yields of formic acid from the 4-hexosido-glucoses are higher than those obtained from the 6-hexosido-glucoses. Since the formation of 3-glucosido-arabinose from cellobiose and maltose is accompanied by hydroxymethylene formation, the relative amount of formic acid obtained from the pentose derivative should, in general, be less than that from the disaccharides. In Fig. 5 a comparison is shown of the yields of formic acid obtained from maltose, cellobiose and 3-glucosido-arabinose. At approximately 3.2 N and 2 N potassium hydroxide it is seen that the yields of formic acid from maltose and cellobiose, respectively, tend toward lower values than those obtained from the glucosido hepta-acetate. This unexpected
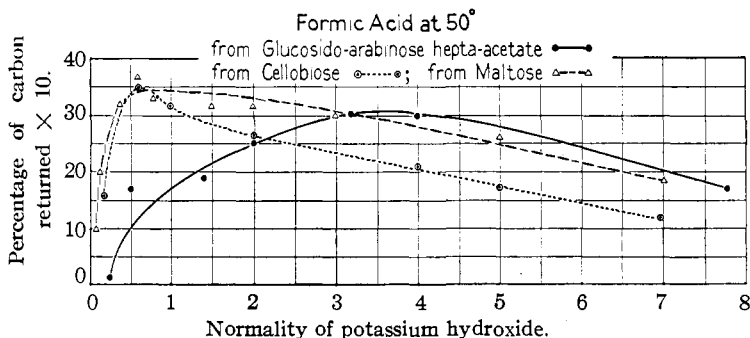

Fig. 5.

behavior in the region of higher alkalinities may be due to an equilibrium condition whereby the two well-known disaccharides now begin to form appreciable amounts of their 2,3-enediol which on rupture at the double bond would yield glycol aldehyde and 2-glucosido-erythrose. This shifting in the alkaline system would tend to lower the concentration of hydroxymethylene molecules, and hence the formic acid yields. By reason of the hindering action of the hexosido section of the molecule, the glucosido-arabinose cannot form a 2,3-enediol and hence its alkaline decomposition up to the point of hydrolysis must yield hydroxymethylene continuously. The fact that alkaline solutions of glycol aldehyde of sufficient molar strength will also yield formic acid must not be overlooked in seeking a sound explanation for the above behavior of glucosido-arabinose hepta-acetate.

Owing to the extreme complexity of the reaction mixtures in each of these cases it has been found difficult to determine quantitatively the small amounts of formic acid in the presence of so much acetic acid, with an accuracy such as we would desire. Hence, these data with reference to formic acid are valuable as indicating the tendency of the reaction.

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