3 ml. of concd. hydrochloric acid and extracted several times with ether. The combined ethereal extracts were washed, dried and concentrated. Distillation of the residual oil gave a fraction, b.p. 135–140° (7 mm.), which was recrystallized from petroleum ether to afford 1.8 g. of VIa′ as colorless plates, m.p. 72.5–73.5°, $[\alpha]_D^{27}$ −14° (*c* 1.6, ethanol). For infrared and NMR properties see the discussion part. The 2,4-dinitrophenylhydrazone melted at 197° after recrystallizations from ethanol.

Anal. Calcd. for $C_{21}H_{26}N_4O_4$: C, 63.30; H, 6.58. Found: C, 63.29; H, 6.78.

Autoxidation of the α,β-unsaturated aldehyde (VIa′); *preparation of the α,β-unsaturated acid* (VII). When 1.4 g. of the α,β-unsaturated aldehyde (VIa′) was kept in an open vessel at room temperature for 2 weeks, the crystals first liquefied and then resolidified. Recrystallizations from petroleum ether afforded 1.0 g. of colorless prisms melting at 150–150.5°. Infrared absorptions were given above.

Anal. Calcd. for $C_{15}H_{22}O_2$: C, 76.88; H, 9.46. Found: C, 76.78; H, 9.59.

KYÔTO, JAPAN

[CONTRIBUTION FROM THE RESEARCH LABORATORY OF THE A. E. STALEY MANUFACTURING CO.]

Reactions of Sugars in the Presence of Acids: a Paper Chromatographic Study

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Three percent solutions of D-glucose, D-mannose, D-galactose, D-fructose, L-sorbose, maltose and sucrose were heated in 0.05*N* to 4.0*N* hydrochloric acid at 98° for various lengths of time. The reactions (furan ring formation, condensation, hydrolysis) were followed by paper chromatography. D-Glucose showed the greatest tendency to form reversion products, followed in decreasing order by D-galactose and D-mannose. D-Fructose and L-sorbose showed no formation of reversion products. No monomolecular anhydro sugar formation was observed for any of the sugars. Sucrose hydrolyzed rapidly; the resulting mixture of D-glucose and D-fructose did not form condensation products of D-glucose with D-fructose. Maltose hydrolyzed slowly enough to co-exist with reversion products of D-glucose.

Many workers have reported the general finding that sugars in solution exposed to acidic environments form anhydro sugars, furan derivatives and condensation products.[1–3] D-Altrose forms an anhydro sugar in 57% yield[4]; D-glucose, treated in analogeous manner, produces 1,6-anhydro-β-D-glucopyranose in trace quantities only.[5] The chief factor deciding the stability of 1,6-anhydropyranoses is believed to be steric strain due to repulsion between substituents in the chair configuration.[6] Heating with oxalic acid under pressure converts 54% of D-fructose or of the D-fructose moiety of sucrose into 5-hydroxymethyl-2-furaldehyde.[7,8] The D-glucose moiety of the molecule can be isolated almost quantitatively from the reaction. 5-Hydroxymethyl-2-furaldehyde is formed in only small percentage yield from D-glucose.[9,10]

The condensation of sugars under conditions generally prevailing during the acid hydrolysis of carbohydrates is termed reversion. Reversion of D-glucose has been investigated mainly, but other sugars have also been considered. Reversion products from D-glucose have been isolated chromatographically and identified as their crystalline acetates.[5,11] The reversion products from D-glucose consist mainly of β-isomaltose and β-gentiobiose besides trace products including seven other disaccharides and 1,6-anhydro-β-D-glucopyranose. A mixture of oligosaccharides results from the interaction of D-galactose, D-mannose or L-arabinose and hydrochloric acid at room temperature.[12,13] Transglycosidation products have been obtained when solutions of various single disaccharides or mixtures of sugars are heated in a boiling water bath in the presence of acetic, hydrochloric, or sulfuric acid.[14] Concentration by rapid evaporation of solutions of D-glucose and other simple sugars in hydrochloric acid yielded condensation products.[3] Hexose condensation products were prepared by the action of gaseous hydrogen chloride on D-glucose, maltose, D-galactose and lactose.[14a,15]

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(13) T. K. N. Jones and W. H. Nicholson, *J. Chem. Soc.*, 27 (1958).
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(15) C. R. Ricketts and C. E. Rowe, *J. Chem. Soc.*, 3809 (1955).

A disaccharide was isolated as a reaction product of D-fructose and dry gaseous hydrogen chloride.[16] The catalytic influence of thionyl chloride,[17] phosphorous acid,[18] and metaboric acid[19] on the formation of condensation products has also been reported. However, no instance has been found in the literature where anhydro sugar development, furan ring formation and condensation have been systematically studied together.

The present work reports on the comparative behavior of various sugars participating in these three types of reactions. Since the conversion of monosaccharides into oligosaccharides is reversible, the hydrolysis of disaccharides has also been studied and the rate compared with the rate of condensation of monosaccharides.

In the present study no monomolecular anhydro sugar formation was observed from reactions involving either aldoses or ketoses. A pure sample of 1,6-anhydro-β-D-glucopyranose was used to locate the region where anhydro hexoses could be expected on the chromatograms: no spots were obtained in this region with the acid treated sugar solutions. Diketohexose dianhydrides are believed to have formed in trace amounts from D-fructose and L-sorbose; three very faint spots of non-reducing carbohydrates were obtained in the region where these products could be expected on the chromatograms.

The solutions of the ketoses became yellow faster and formed insoluble humin-like products to a greater extent than did the solutions of aldoses. Furan ring formation was observed only with the ketoses D-fructose and L-sorbose, but not with the aldoses. A pure sample of 5-hydroxymethyl-2-furaldehyde was used to locate its place on the chromatogram. Spots obtained with reaction products from D-fructose and L-sorbose coincided in position and color with the spot obtained from pure 5-hydroxymethyl-2-furaldehyde.

All the aldoses formed reversion products. The tendency among the aldoses investigated to form oligosaccharides was greatest with D-glucose, less with D-galactose, and still less with D-mannose; more oligosaccharides were formed from D-glucose than from the two other aldoses; they were also formed faster. The ketoses D-fructose and L-sorbose did not form reversion products.

Differences in the rate of acid hydrolysis of disaccharides result in a faster or slower release of monosaccharides; this can be expected to modify the nature of the condensation of the released monosaccharides. For example, sucrose hydrolysis proceeded at a rate much faster than the rates of either furan ring formation or condensation. No formation of new condensation products between D-glucose and D-fructose was observed. Maltose was found to hydrolyze slowly enough to exist among newly formed condensation products of D-glucose.

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EXPERIMENTAL

Test tubes (Pyrex) containing 3% solutions of sugars in hydrochloric acid were plunged into a steam bath at 98°. At regular time intervals samples were withdrawn from the test tubes and cautiously neutralized with calcium carbonate or with strongly basic ion exchange resin. The neutralized solutions were spotted on Whatman paper 54 (fast flow rate, high wet strength). The solvent used was 1-butanol-acetic acid–methanol-water, 517-100-217-166, forming a single phase. The descending technique was used for 12 to 72 hr. After irrigation, the dry paper was drawn through a silver nitrate reagent[20] (2 g. of silver nitrate dissolved in 5 ml. of water, added to 500 ml. of acetone) dried, drawn through an alkaline reagent (5 ml. of 25% sodium hydroxide added to 500 ml. of 95% ethanol), dried, exposed to live steam, immersed in 2*N* ammonium hydroxide, washed for 3 hr. in running water, immersed in a 1% aqueous solution of sodium hydrosulfide, washed for 3 hr. in running water and dried. The black spots on the white background are permanent and easily recorded photographically. The application of live steam greatly increases the sensitivity. Non-reducing carbohydrates which would ordinarily not appear, or would appear only faintly, are detected by the combined application of heat and humidity. Quantities as low as 0.2 μg. of D-glucose, 5 μg. of sucrose or 5 μg. of raffinose were detected by this procedure. Identification of the sugar spots on the paper was based on simultaneous runs of known sugars and comparison of their respective positions on the chromatograms. 5-Hydroxymethyl-2-furaldehyde was detected with thymol-aniline–phosphoric acid reagent.[21]

D-Glucose was heated in 1.0*N* hydrochloric acid for 120 min. and in 4.0*N* hydrochloric acid for 30 min. After a 10 min. treatment at 98° with 4.0*N* hydrochloric acid, the D-glucose solution became straw colored, after 30 min. yellow, and after 60 min. brownish. No precipitate was formed. Reversion products[5] formed extensively. Disaccharides united by a 1 → 6 linkage, isomaltose or gentiobiose or both, are formed first. They move more slowly than maltose on the paper chromatograms. Somewhat later disaccharides appear at the positions of maltose (1 → 4 linkage) and nigerose (1 → 3 linkage), followed by the appearance of trisaccharides. Tetrasaccharides were detected after 2 hr. treatment with 1.0*N* hydrochloric acid or 10 min. with 4.0*N* hydrochloric acid. D-Glucose is still present in the solution in large amounts after treatment with 4.0*N* hydrochloric acid for 30 min.

D-Mannose was heated in 1.0*N* hydrochloric acid for 85 min. and in 4.0*N* hydrochloric acid for 122 min. The D-mannose solution stayed colorless up to 30 min. heating with 1.0*N* hydrochloric acid, became pale straw colored after 50 min. and straw colored after 85 min. The 4.0*N* hydrochloric acid solution was brown after 122 min. heating and contained a dark precipitate of humin-like products. Only traces of reversion products were formed: two very faint spots appeared in the disaccharide region upon treatment with 1.0*N* hydrochloric acid but they did not change appreciably in intensity upon increasing the heating time. Only one faint spot, coinciding in position with maltose on the paper chromatogram, appeared upon treatment with 4.0*N* hydrochloric acid. It did not change in intensity with increasing heating time.

(20) Silver nitrate is not a very specific reagent, but it is very sensitive.

(21) T. E. Courtcois, A. Wickström, P. Fleury, and P. LeDizet, *Bull. soc. chim. biol.*, **37**, 1013 (1955).

D-Galactose behaved like D-mannose with regard to color formation. D-Galactose had a more pronounced tendency to form reversion products than D-mannose: one spot in the disaccharide region and another in the trisaccharide region appeared on the paper chromatogram upon heating for 1 min. at 98° in 1.0*N* hydrochloric acid. After 30 min. heating the two spots were still faint; after 50 min. they appeared stronger, and after 87 min. higher molecular weight reversion products were also detectable on the paper chromatograms. Di- and trisaccharide spots appeared faintly on the paper chromatograms after heating D-galactose with 4.0*N* hydrochloric acid 126 min.

D-Fructose was heated with 0.1*N* hydrochloric acid and 4.0*N* hydrochloric acid for up to 120 min. Although the formation of brown colored, solid resinous products occurred very early, D-fructose was still present in solution after 30 min. treatment with 4.0*N* hydrochloric acid but was completely destroyed after 60 min. D-Fructose did not produce any reversion product. The chromatogram revealed a spot corresponding to 5-hydroxymethyl-2-furaldehyde. Three very faint spots were revealed on the chromatograms following the steam treatment. They are located between the dextrose and the maltose spot and appear only in the reaction with the lower concentration of hydrochloric acid. The spots are attributed to bimolecular fructose anhydrides in very low concentration.

L-Sorbose was treated with 0.1*N* hydrochloric acid up to 75 min. and with 4.0*N* hydrochloric acid up to 30 min. L-Sorbose was still detected after 20 min. treatment with 4.0*N* hydrochloric acid but not after 30 min. No formation of reversion products was observed but 5-hydroxymethyl-2-furaldehyde and very minor amounts of three compounds, believed to be bimolecular sorbose anhydrides, were present. L-Sorbose behaved in this respect like the other ketose, D-fructose.

Maltose was heated with 0.1*N* hydrochloric acid up to 60 min. and with 0.5*N* hydrochloric acid up to 30 min. Maltose could still be detected after 30 min. treatment with 0.5*N* hydrochloric acid. During the time the degradation of maltose into two molecules of D-glucose took place, and before all the maltose was hydrolyzed, reversion products were formed.

Sucrose was treated in 0.05*N* hydrochloric acid up to 30 min. and in 1.0*N* hydrochloric acid up to 40 min. The chromatograms showed that the sucrose was hydrolyzed completely into D-glucose and D-fructose after 5 min. in 0.05*N* hydrochloric acid and before the appearance of reversion products. Prolonged treatment with hydrochloric acid produces reversion products. The reversion products occupy the same positions on the chromatograms as the reversion products obtained with D-glucose alone. Their presence is attributed to the glucose moiety of sucrose.

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DECATUR, ILL.

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH PUBLIC HEALTH SERVICE, U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE]

2-Deoxy-D-ribose. VI.[1] The Preparation of Derivatives of 3-Deoxy-D-*ribo*-hexonic Acid and 3-Deoxy-D-*arabino*-hexonic Acid Therefrom. Some Observations on the Kiliani Synthesis

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Derivatives of 3-deoxy-D-*ribo*-hexonic acid and 3-deoxy-D-*arabino*-hexonic acid are most easily prepared in pure form through the addition of hydrogen cyanide to 2-deoxy-D-ribose, the total yield being 80%. A variety of substances derived from these acids, including 3-deoxy-D-*ribo*-hexitol and 3-deoxy-D-*arabino*-hexitol, are described. With 2-deoxy-D-ribose and D-ribose in the usual Kiliani synthesis, hydrolysis of the epimeric nitriles is spontaneous and rapid, requiring no special step in the procedure.

The 3-deoxyhexoses, 3-deoxy-D-*ribo*-hexose (3-deoxy-D-glucose), and 3-deoxy-D-*arabino*-hexose (3-deoxy-D-mannose), as well as some of their derivatives, are of biochemical as well as of theoretical interest. Unfortunately, however, the synthetic methods which have been used for the preparation of these two substances[2,3] are of such complexity and length as to render the sugars themselves relatively inaccessible. In the course of the present work we have investigated some alternative and potentially simpler synthetic pathways to these two 3-deoxyhexoses.

In 1910 J. U. Nef[4] noted that among the saccharinic acids produced by the isomerization of D-glucose[5] with a large excess of hot sodium hydroxide were the two 3-deoxyhexonic acids, 3-deoxy-D-*ribo*-hexonic acid ("α-D-glucometasaccharinic acid") and 3-deoxy-D-*arabino*-hexonic acid ("β-D-glucometasaccharinic acid"). Using the mixture produced according to Nef's directions, Sowden[6] carried the process one step further with a Ruff degradation to

(1) 2-Deoxy-D-ribose. V: C. Pedersen and H. G. Fletcher, Jr., *J. Am. Chem. Soc.*, **82**, 5210 (1960).

(2) Crystalline 3-deoxy-D-glucose: J. W. Pratt and N. K Richtmyer, *J. Am. Chem. Soc.*, **79**, 2597 (1957).

(3) H. R. Bolliger and D. A. Prins, *Helv. Chim. Acta*, **29**, 1061 (1946); crystalline 3-deoxy-D-mannose: G. Rembarz, *Chem. Ber.*, **93**, 622 (1960).

(4) J. U. Nef, *Ann.*, **376**, 1 (1910).

(5) These saccharinic acids, being deoxyhexonic acids ($C_6H_{12}O_6$), are, formally, isomers of D-glucose ($C_6H_{12}O_6$).