stubby needles, dec. 156–157° (gas) colors 150° (preheat 145°); $[\alpha]^{26.5}D$ (pyridine) +147° (0 time) → +121° (const. 24 hours); $[\alpha]^{25}D$ (0.1 *N* HCl) +103.7° (21 min.) → +98.7° (const. 2 hours); (*c* 2, , *l* 2.0) calcd. as hydrochloride, $[\alpha]D$ +84.5° (equilibrium).

Hydrochloride of the Isopropylamine-Fructose Condensation Product.—This derivative was prepared by the same procedure as the hydrochloride of the cyclohexylamino condensation product. It was obtained as coarse colorless prisms when recrystallized from methanol–acetone (1/3) at −20°, dec. (gas) 185–186° (preheat to 150°).

Anal. Calcd. for $C_9H_{19}NO_5HCl$: Cl, 13.33; N, 5.44. Found: Cl, 13.7; N, 5.41; $[\alpha]^{25}D$ +100° → +84.7 (const. 2 hours) [*c* 1.2 *l* 2 (water or 0.1 *N* hydrochloric acid)].

The mutarotation curves are shown in Figs. 1 and 2. Evidence for the purity of the hydrochloride and of the free base was obtained by fractional crystallization. Fractional crystallization of the isopropylamine condensation product from ethanol yielded 4 fractions with the equilibrium rotations in 0.1 *N* hydrochloric acid of +99.1, 99.2, 99.0 and 98.1. The hydrochloride of the original sample was separated into three fractions by adding various amounts of acetone to the methanol solution. The isolated salts had similar mutarotation curves with the equilibrium rotations, +84.7, 84.5 and 84.8. Attempts to obtain a crystalline anomeric form of the free base or of the hydrochloride by varying conditions of crystallization have not been successful.

Preparation of Phenyl-D-glucosazone from 2-Cyclohexylamino-2-deoxyaldohexose.[18]—Eight hundred mg. of 2-cyclohexylamino-2-deoxy aldose hydrochloride, 1.5 g. of phenylhydrazine hydrochloride, 2.5 g. of sodium acetate and 0.5 g. of sodium bisulfite in 30 ml. of water was refluxed for 6 hours. A precipitate was observed after 4 hours. The mixture was cooled and the yellow-brown solid filtered and washed with acetone to remove resinous material. The filtrate was refluxed again for two 6-hour periods, filtering and washing with acetone each time. A total of 99 mg. (10%) of phenyl-D-glucosazone was obtained. On recrystallization from methanol–water, the derivative melted at 207–208°. The identity of the compound as phenylglucosazone was confirmed by the preparation of the phenyl-D-glucosotriazole, m.p. 194–195°, by the procedure of Hann and Hudson.[19]

Acknowledgments.—The author gratefully acknowledges the assistance of L. M. White and G. E. Secor for elementary analyses and F. T. Jones for microscopic examinations.

(18) Based on procedure of F. A. Kuehl, Jr., E. H. Flynn, F. W) Holly, R. Mozingo and K. Folkers (THIS JOURNAL, **69**, 3032 (1947). for conversion of N-methyl-L-glucosamine to phenyl-L-glucosazone.

(19) R. M. Hann and C. S. Hudson, *ibid.*, **66**, 735 (1944); **68**, 1769 (1946).

ALBANY, CALIFORNIA

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY]

2-*O*-α-D-Xylopyranosyl-L-arabinose from Hemicellulose-B of Corn Cob[1,2]

BY ROY L. WHISTLER AND D. I. MCGILVRAY

RECEIVED OCTOBER 7, 1954

Mild acid hydrolysis of corn cob hemicellulose-B yields a number of neutral oligosaccharides which are distinct from the β-D-1 → 4 xylobiose polymer homologous series. One of these oligosaccharides has been isolated and characterized as 2-*O*-α-D-xylopyranosyl-L-arabinose.

In work toward elucidating the structure of hemicellulose-B of corn cob considerable reliance is placed on the method of fragmentation analysis, wherein the polysaccharide is hydrolyzed to oligosaccharides whose structures can be determined exactly. The nature of adjoining monosaccharide units and the glycosidic linkages between them thereby becomes fixed with a high degree of certainty. This method for the determination of polysaccharide structures is of great assistance in dealing with complex molecules.

From the hemicellulose-B of corn cob several acidic oligosaccharides have already been characterized after isolation from the polysaccharide partial hydrolyzate.[3–5] Attention is now turned to neutral oligosaccharides. These are produced in highest yield when hemicellulose-B is subjected to mild hydrolysis such as by dilute sulfuric acid at room temperature, or by autolysis through boiling, in water, the naturally acidic hemicellulose. Neutral oligosaccharides so obtained are not members of the β-D-1 → 4 xylobiose polymer homologous series. They are readily isolated by successive charcoal[6] and cellulose[7] column chromatography.

One of the neutral disaccharides, isolated in 1.5% yield, is described here. It reduces Fehling solution and hydrolyses to D-xylose and L-arabinose. On oxidation with bromine water, followed by hydrolysis and paper chromatography, only D-xylose is found. Thus L-arabinose is at the reducing end of the molecule. Since no osazone is isolated after reaction of the disaccharide with phenylhydrazine acetate a 1 → 2 linkage is indicated. Methylation with dimethyl sulfate, first at 0° then at higher temperatures produces a fully methylated product which on hydrolysis gives rise to 2,3,4-tri-*O*-methyl-D-xylose, 3,5-di-*O*-methyl-L-arabinose and 3,4-di-*O*-methyl-L-arabinose. From this it is concluded that the linkage is 1 → 2 and that the initial low temperature methylation caused the fixation in the L-arabinose portion of a mixture of furanoside and pyranoside structures. The positive rotation (+39.8°) of the disaccharide suggests that the glycosidic linkage is α-D. Thus the structure 2-*O*-α-D-xylopyranosyl-L-arabinose may be proposed. This disaccharide is not produced when a mixture of D-xylose and L-arabinose are subjected to the hydrolytic conditions used in the depolymerization of hemicellulose-B.

(1) Journal Paper No. 819 of the Purdue Agriculture Experiment Station.

(2) Paper presented before the Division of Carbohydrate Chemistry at the 126th Meeting of the American Chemical Society at New York, N. Y., September, 1954.

(3) R. L. Whistler and L. Hough, THIS JOURNAL, **75**, 4918 (1953).

(4) R. L. Whistler, H. E. Conrad and L. Hough, *ibid.*, **76**, 1668 (1954).

(5) R. L. Whistler and D. I. McGilvray, *ibid.*, in press.

(6) R. L. Whistler and D. F. Durso, *ibid.*, **72**, 677 (1950).

(7) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, 2511 (1949).

3,4-Di-O-methyl-L-arabolactone and its derived amide are described here for the first time.

Experimental

Paper Chromatography.—Separations were made on Whatman No. 1 filter paper at 25° by the descending method,[8] using one of the following solvents in the volume ratios indicated: (A) butanol-1–ethanol–water (40:11:19, v./v.), (B) ethyl acetate–pyridine–water (8:2:1, v./v.), (C) methyl ethyl ketone 90% saturated with water. *p*-Anisidine hydrochloride[9] was used as spray to reveal the positions of the sugars on the chromatograms.

Partial Hydrolysis.—Hemicellulose-B[10] (100 g.) was dissolved in 2 l. of *N* sulfuric acid and allowed to stand at room temperature for 3 weeks, at the end of which time the oligosaccharide concentrations appeared to have reached a maximum as judged by periodic examination of portions of the neutralized hydrolyzate on paper chromatograms. Barium hydroxide solution was added to *p*H 7 and after filtration, the filtrate was poured, with stirring, into 9 l. of 95% ethanol. The degraded hemicellulose (76 g.) was collected on the centrifuge and the centrifugate evaporated to dryness under reduced pressure. An aqueous solution of the alcohol soluble material was introduced on to a carbon–Celite column[6] (65 mm. × 600 mm.) and the column washed with 16 l. of water. This aqueous effluent contained monosaccharides only. The 5% ethanol fraction (12 l.) contained two major compounds, $R_{xylobiose}$[11] 1.20 and 0.83. Two further oligosaccharides, $R_{xylobiose}$ 0.64 and 0.40 (xylotriose) were eluted with 10% ethanol.

The 3.3 g. of material eluted by 5% ethanol was further fractionated on a column of powdered cellulose[7] (50 mm. × 500 mm.) using butanol-1 saturated with water. The first oligosaccharide to be eluted (1.5 g.; $R_{xylobiose}$ 1.20) had $[\alpha]^{25}D$ +39.8° (*c* 2.8 in water). It is a hygroscopic powder and moves as a single component with chromatographic solvents (A) and (B).

Characterization of the Disaccharide.—Hydrolysis of a 10-mg. sample with 1 ml. of *N* sulfuric acid at 100° for 3 hours in a sealed tube, followed by neutralization with barium carbonate and subsequent paper chromatography (solvents (A) and (B)) showed the presence of xylose and arabinose only. A further 21-mg. sample was oxidized with bromine water at room temperature for 4 days and recovered in the usual manner. Hydrolysis and paper chromatography of the oxidized disaccharide showed the presence of xylose only.

The disaccharide (47 mg.), water (0.75 ml.), phenylhydrazine (0.1 ml.) and acetic acid (0.1 ml.) were heated at 80° for 3 hours. No osazone separated on cooling and standing at 0° for some days. On addition of 2 ml. of water the solution was extracted exhaustively with ether. The aqueous solution was made normal with respect to sulfuric acid, then hydrolyzed, neutralized and chromatographed as described above. Xylose and arabinose were detected in approximately equal concentrations.

Disaccharide (770 mg.) was dissolved in water (10 ml.) containing dimethyl sulfate (7 ml.). Sodium hydroxide solution (12 ml., 30%) was added dropwise over 6 hours with vigorous stirring and external ice cooling. After stirring overnight sodium hydroxide (12 ml., 30%) was added followed by the dropwise addition of dimethyl sulfate (7 ml.) at room temperature. The stirring was maintained for 16 hours at the end of which time similar quantities of sodium hydroxide solution and dimethyl sulfate were added in like manner. The stirring was again continued overnight and then the mixture was brought to *p*H 5–6 by the addition of glacial acetic acid. The solution was extracted continuously with chloroform for 24 hours. Evaporation of the chloroform under reduced pressure left a sirup (1.023 g.) which was twice methylated with silver oxide (4 g.) and methyl iodide (10 ml.) to yield the fully methylated disaccharide (977 mg.).

Hydrolysis was effected with sulfuric acid (25 ml., *N*) at 95° for 2 hours (constant rotation). After neutralization with barium carbonate, filtration and evaporation, a clear sirup (854 mg.) was obtained. Examination on the paper chromatogram showed the presence of 3 components I, II and III, R_G values,[12] in solvent (A) 0.96, 0.85 and 0.06, in solvent (C) 0.97, 0.86 and 0.49, respectively. The components were separated[13] on sheets on filter paper using solvent (C).

Component I (253 mg.) after recrystallization from ether–petroleum ether had $[\alpha]^{25}D$ +15° (*c* 1.1 in water) and m.p. 91°. A mixed m.p. with 2,3,4-tri-O-methyl-D-xylose was unchanged. The X-ray diffraction patterns of this material and authentic 2,3,4-tri-O-methyl-D-xylose were identical.

Anal. Calcd. for $C_8H_{16}O_5$: C, 50.0; H, 8.4. Found: C, 49.92; H, 8.47.

Component II (74.8 mg.), a sirup, had $[\alpha]^{25}D$ −15.3° (*c* 1.3 in water). The R_g value in solvent (A) approximated closely to that of 2,5-di-O-methyl-L-arabinose.[14] The sample was oxidized with bromine water for 4 days at room temperature and the lactone (70 mg.) recovered as usual. This crystallized and had m.p. 74°. A m.p. of 60° is recorded[15] for 2,5-di-O-methyl-L-arabolactone, 78° for that of 3,5-di-O-methyl-L-arabolactone.[16]

Anal. Calcd. for $C_7H_{12}O_6$: C, 47.8; H, 6.84. Found: C, 47.5; H, 6.86.

This lactone (15 mg.) treated with phenylhydrazine (6 mg.) in ether (1 ml.) at room temperature overnight yielded a crystalline phenylhydrazide which was shown by X-ray diffraction not to be 2,5-di-O-methyl-L-arabonic acid phenylhydrazide. About 20 mg. of lactone was dissolved in 2 ml. of methanol saturated with ammonia and allowed to stand at 0° overnight. Removal of the solvent gave the amide which on recrystallization from acetone had m.p. 143°. Hirst, Jones and Williams[16] record m.p. 145° for 3,5-di-O-methyl-L-arabonamide. The X-ray diffraction pattern of this amide was identical with that of an authentic specimen of 3,5-di-O-methyl-L-arabonamide.

Component III (189 mg.), a sirup, had $[\alpha]^{25}D$ +125° (*c* 1.6 in water). On paper chromatograms developed with solvents (A) and (C) it corresponded in position with a known sample of 3,4-di-O-methyl-L-arabinose. This sugar derivative was oxidized with bromine water for 4 days at room temperature to yield a sirupy lactone, $[\alpha]^{25}D$ +44.0° → −1.0° (equilibrium in 6 hr.), (*c* 2.68 in water). A sample of the lactone was converted to the crystalline amide, as described above, which, after recrystallization from acetone had m.p. 133° $[\alpha]^{25}D$ +28.2°.

Anal. Calcd. for $C_7H_{15}O_5N$: C, 43.5; H, 7.7. Found: C, 43.44; H, 7.68.

Authentic 3,4-di-O-methyl-L-arabinose was prepared by methods previously described.[14,17] This was converted into the corresponding amide, as above. A mixed m.p. with the amide prepared from component III was unchanged. In addition the X-ray diffraction patterns of the two amides were identical.

Test for Reversion.—Chromatographically pure samples of D-xylose and L-arabinose (1 g. of each) were treated under the conditions described for the hydrolysis of hemicellulose-B. No trace of reversion products could be detected after neutralization of the acid and subsequent paper chromatography.

Acknowledgment.—We wish to thank Dr. J. K. N. Jones for samples of 2,5-di-O-methyl-L-arabonic acid phenylhydrazide, 3,5-di-O-methyl-L-arabonamide and 3,4-di-O-methyl-L-arabinose.

LAFAYETTE, INDIANA

(8) S. M. Partridge, *Biochem. J.*, **42**, 238 (1948).

(9) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, 1702 (1950).

(10) R. L. Whistler, J. Bachrach and D. R. Bowman, *Arch. Biochem.*, **19**, 25 (1948).

(11) Rate of movement relative to α-D-1 → 4 xylobiose ($R_{xylobiose}$) 1.0.

(12) E. L. Hirst, L. Hough and J. K. N. Jones, *J. Chem. Soc.*, 928 (1949).

(13) A. E. Flood, E. L. Hirst and J. K. N. Jones, *ibid.*, 1679 (1948).

(14) P. Andrews, D. H. Ball and J. K. N. Jones *ibid.*, 4090 (1953).

(15) F. Smith, *ibid.*, 1035 (1940).

(16) E. L. Hirst, J. K. N. Jones and E. M. L. Williams, *ibid.*, 1062 (1947).

(17) J. Honeyman, *ibid.*, 990 (1946).