Periodate Oxidation.—The above compound, obtained by reductive desulfurization, in a concentration of 0.0025 *M* was oxidized with 0.04 *M* sodium metaperiodate at 25° in the dark. Samples were taken at intervals and the periodate consumed was determined by the arsenite method. Formic acid was estimated, after destruction of excess periodate with ethylene glycol, by titration with 0.01 *M* sodium hydroxide with phenolphthalein as indicator. The absence of formaldehyde and acetaldehyde was shown by no reaction with dimedone.

HALIFAX, N. S., CANADA

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH]

D-*glycero*-D-*allo*-Heptose, L-*allo*-Heptulose, D-*talo*-Heptulose and Related Substances Derived from the Addition of Cyanide to D-Allose[1]

BY JAMES W. PRATT AND NELSON K. RICHTMYER

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The addition of cyanide to D-allose (I) has led to the production of D-*glycero*-D-*allo*-heptose (VI) and its non-crystalline epimer V. Hydrogenation of these aldoheptoses and oxidation of the heptitols thus obtained by *Acetobacter suboxydans* yielded L-*allo*-heptulose (X) and D-*talo*-heptulose (IX). D-*allo*-Heptulose (IV) was prepared by the rearrangement in alkali of the epimeric aldoheptoses.

Oxidation by *Acetobacter suboxydans* of heptitols which have the favorable D-*erythro* configuration for the terminal triol group has been utilized in this Laboratory to prepare L-*galacto*-heptulose (perseulose), L-*gluco*-heptulose, D-*manno*-heptulose, D-*altro*-heptulose (sedoheptulose), L-*gulo*-heptulose and D-*ido*-heptulose.[2] We now wish to report that the procedure has been successfully employed to obtain the last remaining heptuloses which can be prepared in this manner, *viz.*, crystalline L-*allo*-heptulose (X) from *meso*-*glycero*-*allo*-heptitol (VIII) and crystalline D-*talo*-heptulose (IX) from the epimeric D-*glycero*-D-*altro*-heptitol (synonym, D-*glycero*-L-*allo*-heptitol) (VII). Neither these ketoses nor their enantiomorphs have been obtained previously. We have thus completed the catalog of heptulose configurations[3] and have reconfirmed the *Acetobacter* specificity rule of Bertrand as extended to *A. suboxydans* by Hann, Tilden and Hudson. Our co-equal interest in these sugars—their behavior in hot, aqueous acid—will be considered in a subsequent publication.[4]

The addition of hydrogen cyanide to D-allose followed by the usual hydrolysis yielded a pair of epimeric acids which were conveniently separated as their lactones when one of the epimers was found to crystallize readily. The phenylhydrazide of the temporarily non-crystalline lactone was prepared from the mother liquor, and this compound likewise was obtained epimerically pure after one or two recrystallizations.

A phenylhydrazide was prepared from the crystalline (*"allo"*) lactone III in the usual manner in order that the "phenylhydrazide rule" might be applied. The (*"altro"*) phenylhydrazide which was obtained originally was decomposed to yield the corresponding lactone II. This compound likewise crystallized. Thus it appears that the fortuitous separation of epimers is not necessarily to be expected on repetition of this procedure.

In order to assign the correct configuration to the lactones we adduce the evidence and arguments which follow. The lactone which first crystallized—D-*glycero*-D-*allo*-heptonolactone—showed $[\alpha]^{20}D$ +3.9°, while its epimer showed $[\alpha]^{20}D$ +26.3°. Hudson[5] calculated the values −10 and +26°, respectively, for these lactones. More significantly, the phenylhydrazides showed $[\alpha]^{20}D$ (*"allo"*) +16.7° and (*"altro"*) −22°; these values justify our assignment of configuration at C2 in accordance with the "phenylhydrazide rule."[6] Finally, sodium amalgam reduction of the *"allo"* lactone yielded a crystalline sugar (VI) which was further reduced to an optically inactive (and therefore *meso*-*glycero*-*allo*-) heptitol (VIII).

The preparation of D-*allo*-heptulose (IV) is announced because of some interest in the compound. A fuller report is planned.

Experimental

D-*glycero*-D-*allo*-**Heptono-γ-lactone (III).**—D-Allose (I)[7] (45 g.) was treated with sodium cyanide (27 g.) using Hudson's modification of the cyanohydrin synthesis.[8,7] After standing at 5° for 20 hours, the solution was boiled to hydrolyze the nitriles and expel ammonia. Sodium ions were removed by passage of the solution through a cation-exchange resin (Amberlite IR-120-H), and the eluate was concentrated *in vacuo* to a thick sirup and then heated *in vacuo* for one hour at 70–80° to effect lactonization. The sirup was dissolved in 120 ml. of absolute ethanol and placed in the refrigerator. After several days 26.6 g. (51%) of crystalline material was obtained. After recrystallization from 8 parts of 95% ethanol, the small, water-white prisms melted at 147.5–148.5° and showed $[\alpha]^{20}D$ +3.94° in water (*c* 8.5) with no evidence of mutarotation.

Anal. Calcd. for $C_7H_{12}O_7$: C, 40.38; H, 5.81. Found: C, 40.47; H, 5.85.

D-*glycero*-D-**allo-Heptonic Phenylhydrazide.**—A solution of 2 g. of the heptonolactone III and 2 ml. of phenylhydra-

(1) Presented in part before the Division of Carbohydrate Chemistry at the New York Meeting of the American Chemical Society, September 17, 1954.

(2) For a short review of this work, as well as pertinent references, see J. W. Pratt N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **74**, 2210 (1952).

(3) With the preparation of D-*allo*-heptulose also announced in this paper, only D-*gulo*, L-*ido*- and L-*talo*-heptuloses remain unknown.

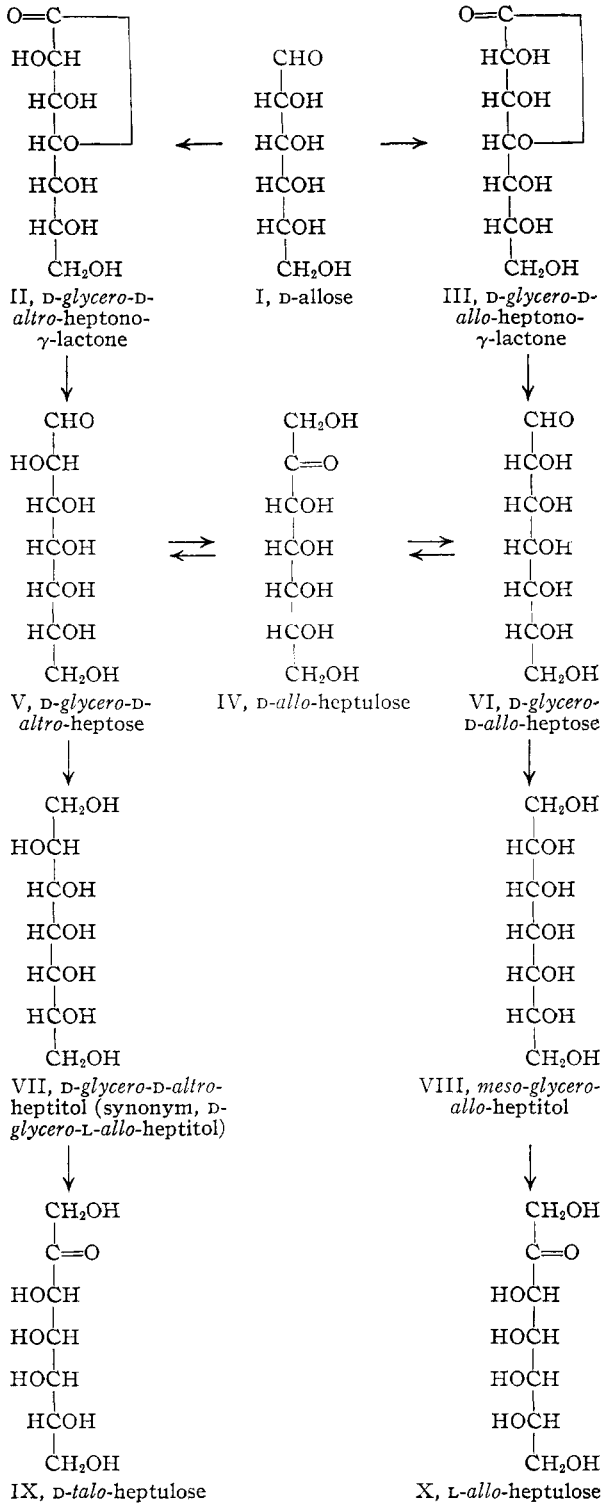
(4) In a preliminary experiment (see Abstracts of Papers, New York Meeting of the American Chemical Society, Sept. 12–17, 1954, p. 22D) we have found that L-*allo*-heptulose is transformed to the extent of about 50% to a non-reducing anhydride which has been characterized as its crystalline tetraacetate (m.p. 116°, $[\alpha]^{20}D$ +46° in chloroform). D-*talo*-Heptulose appears to produce about 10% of an anhydride under the same conditions.

(5) C. S. Hudson, THIS JOURNAL, **61**, 1525 (1939).

(6) P. A. Levene, *J. Biol. Chem.*, **23**, 145 (1915); P. A. Levene and G. M. Meyer, *ibid.*, **31**, 623 (1917); C. S. Hudson, THIS JOURNAL, **39**, 462 (1917).

(7) J. W. Pratt and N. K. Richtmyer, *ibid.*, **77**, 1906 (1955).

(8) C. S. Hudson, *ibid.*, **73**, 4498 (1951).



zine in 5 ml. of water was heated on the steam-bath for two hours, allowed to cool to room temperature and then poured into 20 ml. of absolute ethanol. Crystallization occurred and 2.8 g. of material was obtained as white, prismatic plates; on recrystallization from 95% ethanol the phenylhydrazide melted at 157.5–158.5° with decomposition and showed $[\alpha]^{20}D$ +16.7° in water (*c* 1).

Anal. Calcd. for $C_{13}H_{20}N_2O_7$: C, 49.36; H, 6.37; N, 8.86. Found: C, 49.24; H, 6.43; N, 8.72.

D-*glycero*-D-*altro*-**Heptonic Phenylhydrazide**.—The mother liquor from which the D-*glycero*-D-*allo*-heptono-γ-lactone had separated was concentrated *in vacuo* to a thick sirup (116 g.). A solution of this sirup and 100 ml. of phenylhydrazine in 200 ml. of water was heated for four hours on the steam-bath, and then allowed to stand at room temperature. Crystallization occurred in the form of clusters of needles. After being recrystallized twice from water and twice from aqueous ethanol, the substance was obtained as white, hexagonal platelets which melted at 175.8–176.2° with decomposition and showed $[\alpha]^{20}D$ −21.6° in water (*c* 1.4).

Anal. Calcd. for $C_{13}H_{20}N_2O_7$: C, 49.36; H, 6.37; N, 8.86. Found: C, 49.42; H, 6.24; N, 8.78.

D-*glycero*-D-*altro*-**Heptono-γ-lactone (II)**.—From the phenylhydrazide, decomposed by the method of Hann and Hudson,[9] the corresponding acid was obtained as its γ-lactone (83%): white prisms which melted at 122.5–124° (unchanged by recrystallization) and showed $[\alpha]^{20}D$ +26.5° in water (*c* 8.2). Mutarotation was slight or absent.

Anal. Calcd. for $C_7H_{12}O_7$: C, 40.38; H, 5.81. Found: C, 40.27; H, 5.82.

D-*glycero*-D-*allo*-**Heptose (VI) Monohydrate**.—D-*glycero*-D-*allo*-Heptono-γ-lactone (III) was reduced with sodium amalgam in the presence of sodium hydrogen oxalate.[10] From 25.7 g. of lactone was obtained 20.7 g. (80%) of white needles. The product was dissolved in 0.5 part of hot water, filtered while hot, and twenty parts of boiling absolute alcohol added. The sugar, which crystallized readily when seeded and scratched, melted at 95–98° with subsequent bubbling and showed $[\alpha]^{20}D$ in water +7.20° (3 min.) → +12.68° (24 hr.; constant) (*c* 4.6).

Anal. Calcd. for $C_7H_{14}O_7 \cdot H_2O$: C, 36.84; H, 7.07; H_2O, 7.90. Found: C, 36.82; H, 7.17; loss on drying 4 hr. at 130° under reduced pressure, 8.02.

meso-glycero-allo-**Heptitol (VIII)**.—D-*glycero*-D-*allo*-Heptose monohydrate (16.5 g.) dissolved in water was agitated for 22 hours at 100° in the presence of Raney nickel under hydrogen at 3000 p.s.i. After appropriate treatment the resulting heptitol was obtained as a sirup which crystallized readily from 95% ethanol yielding 12.4 g. (75%) of water-white, prismatic rods; after recrystallization from 90% ethanol, the heptitol melted at 144.5–146° and showed no optical activity in 5% ammonium molybdate solution (*c* 0.5).[11]

Anal. Calcd. for $C_7H_{16}O_7$: C, 39.62; H, 7.60. Found: C, 39.72; H, 7.42.

L-*allo*-**Heptulose (X)**.—A solution of 12 g. of *meso-glycero-allo*-heptitol (VIII) in 600 ml. of water containing 0.5% of Difco yeast extract, 0.3% of potassium dihydrogen phosphate and 0.05% of D-glucose was distributed evenly among three 2-liter erlenmeyer flasks, sterilized by autoclaving for 15 minutes at 120°, inoculated with 1 ml. per flask of a 48-hour culture of *Acetobacter suboxydans*[12] and placed in an incubator at 30°. The reducing activity, estimated by the ferricyanide method of Hagedorn and Jensen as modified by Hanes,[13] reached a maximum in about a week; at that time the product showed a reducing activity about equal to that of D-*manno*-heptulose, based on the assumption of complete conversion of the heptitol. After 13 days the solutions were combined, deproteinized by the method of Somogyi,[14] filtered with carbon, freed of ionic material by passage through Amberlite IR-120 and Duolite A-4 exchange resins and concentrated *in vacuo* to a thick sirup. Crystallization occurred after several days in a vacuum desiccator over anhydrous calcium sulfate, yielding 8 g. of material. After recrystallization from 95% ethanol the sugar melted at 74–76° and showed $[\alpha]^{20}D$ in water −46.6° (20 min.; constant) (*c* 1.5). On drying the sugar at 57° for 4 hours under reduced pressure a loss in weight of 10.39% was observed. If this is applied as a correction the specific rotation becomes $[\alpha]^{20}D$ −52.1°. The dried material melted at 130–132°.

(9) R. M. Hann and C. S. Hudson, THIS JOURNAL, **56**, 957 (1934).

(10) H. S. Isbell, J. V. Karabinos, H. L. Frush, N. B. Holt, A. Schwebel and T. T. Galkowski, *J. Research Natl. Bur. Standards*, **48**, 163 (1952). See also J. W. Pratt, N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **75**, 4505 (1953) (footnote 20).

(11) N. K. Richtmyer and C. S. Hudson, *ibid.*, **73**, 2249 (1951).

(12) American Type Culture Collection No. 621.

(13) H. C. Hagedorn and B. N. Jensen, *Biochem. Z.*, **135**, 46 (1923). C. S. Hanes, *Biochem. J.*, **23**, 99 (1929).

(14) M. Somogyi, *J. Biol. Chem.*, **160**, 69 (1945).

Anal. Calcd. for $C_7H_{14}O_7$: C, 40.0; H, 6.71. Found (dried sample): C, 39.88; H, 6.80.

D-*allo*-**Heptulose (IV).**—D-*allo*-Heptulose was obtained, in small yield, from a mixture of epimeric D-alloheptonolactones by buffered sodium amalgam reduction, followed by alkaline rearrangement of the resulting sirupy mixture of sugars.[15] The sugar appeared to be solvated and so was dried *in vacuo* at 100° for 1.5 hours whereupon it melted at 130–132° and showed $[\alpha]^{20}D$ in water (*c* 0.2) +52.8° (11 min.) with no mutarotation.

Anal. Calcd. for $C_7H_{14}O_7$: C, 40.00; H, 6.71. Found: 39.98; H, 6.67.

D-*glycero*-D-*altro*-**Heptitol (Synonym, D-*glycero*-L-*allo*-Heptitol) (VII).**—Sodium amalgam reduction of D-*glycero*-D-*altro*-heptono-γ-lactone (II) yielded a sirup (V) which failed to crystallize. Raney nickel-catalyzed high-pressure hydrogenation of this sirup produced 11.3 g. (56%) of white, extremely small needles which melted at 125–128° and showed $[\alpha]^{20}D$ −0.3 ± 0.4° in water (*c* 1.2); +53.2° in 5% ammonium molybdate (*c* 0.4); −17.3° in acidified molybdate (*c* 0.32).[11]

(15) W. C. Austin, THIS JOURNAL, **52**, 2106 (1930).

Anal. Calcd. for $C_7H_{16}O_7$: C, 39.62; H, 7.60. Found: C, 39.49; H, 7.62.

D-*talo*-**Heptulose (IX).**—By the procedure reported above for the preparation of L-*allo*-heptulose, 9.5 g. of D-*glycero*-D-*altro*-heptitol (VII) was oxidized with *A. suboxydans*. Estimation of reducing sugar showed a value of 79% of the theoretical maximum in 6 days (assuming the same reducing activity as D-*manno*-heptulose), and this was unchanged on further incubation for 3 days. After appropriate purification the reaction mixture yielded 5 g. (52%) of clusters of prismatic rods. On recrystallization from 90% ethanol the sugar melted at 135–137° and showed $[\alpha]^{20}D$ in water (*c* 1.6) +47.4° (2 min.) → +12.9° (6 hr.; constant).

Anal. Calcd. for $C_7H_{14}O_7$: C, 40.00; H, 6.71. Found: C, 40.02; H, 6.65.

Acknowledgment.—The authors wish to thank Mr. John T. Sipes for the many reductions of lactones with sodium amalgam; Dr. Laura C. Stewart for assistance in the *Acetobacter* oxidations; and Dr. William C. Alford and his associates, all of this Institute, for carrying out the microanalyses.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY]

Oligosaccharides from Partial Acid Hydrolysis of Corn Fiber Hemicellulose[1,2]

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Treatment of corn fiber hemicellulose with mild acid causes the preferential hydrolysis of side chains which consist essentially of L-arabofuranose units. Besides L-arabinose, the hydrolyzate contains 3-*O*-α-D-xylopyranosyl-L-arabinose and L-galactopyranosyl-(1 → 4)-D-xylopyranosyl-(1 → 2)-L-arabinose which are possibly part of the side chains. These two oligosaccharides have been obtained crystalline.

In continuation of the researches of this Laboratory toward the elucidation of the structures of the hemicelluloses, corn fiber hemicellulose has now been examined. This hemicellulose, commercially termed corn fiber gum because of its physical properties, is industrially prepared by lime water extraction of corn hull residues obtained from the wet milling process. Since classical methylation studies only reveal in a general way the types of linkages connecting the various sugar units and not the order of sugar units in a heteroglycan, it is necessary to examine the partial acid hydrolysis products to obtain more complete information on polysaccharide structures.

Corn fiber hemicellulose can be hydrolyzed with normal sulfuric acid at 35°. The hydrolysis proceeds readily with the formation of xylose, arabinose and galactose together with a di- and a trisaccharide. From the rate of hydrolysis it is evident that the structure differs considerably from that of hemicellulose-B of corn cob[3] but is similar to that of the hemicellulose isolated from wheat bran.[4] Paper chromatographic examination of the partial hydrolyzate shows that the oligosaccharides are in highest concentration when the hydrolysis solution attains $[\alpha]^{25}D$ −25° after approximately 24 hr. The polysaccharide isolated in 65% yield from the neutralized hydrolyzate stopped at this stage has a lower arabinose:xylose ratio than the original hemicellulose. Separation of the hydrolyzate into its components is achieved by successive chromatography on columns of charcoal[5] and cellulose.[6] The main component isolated in this way is β-L-arabinose in 11% yield. This, with the recovery of the polysaccharide in high yield, indicated that the prime action of the acid is the hydrolysis of side chains which consist essentially of L-arabinose units, presumably in the furanose form because of their ease of hydrolysis. Further evidence for this is the fact that no uronic acids or oligosaccharides over three sugar units in length are detected by paper chromatography during the partial hydrolysis.

The 5% ethanol eluate from the charcoal column gives in 0.6% yield a crystalline disaccharide which by its lability to alkali is readily differentiated from the 2-*O*-α-D-xylopyranosyl-L-arabinose isolated from hemicellulose-B of corn cob.[3,7] It is degraded by lime water at 25° to a saccharinic acid and xylose, indicating the presence of a 1 → 3 or 1 → 4 linkage. On hydrolysis it gives xylose and arabinose whereas hydrolysis of the bromine oxidized disaccharide gives only xylose. Thus, the disaccharide is a xylosyl-arabinose and analyzes for the monohydrate of such a sugar. The constants are similar to those reported for 3-*O*-α-D-xylopyranosyl-

(1) Journal Paper No. 891 of the Purdue Agricultural Experiment Station, Lafayette, Indiana.

(2) Paper presented before the joint Divisions of Carbohydrate and Cellulose Chemistry at the 128th Meeting of the American Chemical Society at Minneapolis, Minn., September, 1955.

(3) R. L. Whistler and D. I. McGilvray, THIS JOURNAL, **77**, 1884 (1955).

(4) G. A. Adams, *Canad. J. Chem.*, **33**, 56 (1955).

(5) R. L. Whistler and D. F. Durso, THIS JOURNAL, **72**, 677 (1950).

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

(7) R. L. Whistler and W. M. Corbett, THIS JOURNAL, **77**, 3822 (1955).