The Isolation of a Second Octulose and of a Heptose from the Avocado: D-*glycero*-L-*galacto*-Octulose and D-*glycero*-D-*galacto*-Heptose[1]

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In addition to the well known D-*manno*-heptulose and the recently discovered D-*talo*-heptulose and D-*glycero*-D-*manno*-octulose, the avocado contains very small amounts of a second octulose. The new sugar was isolated as an amorphous solid, with $[\alpha]^{20}D$ about $-60°$. Its structure was proved to be D-*glycero*-L-*galacto*-octulose by degradation with lead tetraacetate and with oxygen in alkaline solution, and was confirmed by synthesis, both enzymatic and chemical. Although aldoheptoses have been reported previously as constituents of bacterial polysaccharides, the isolation of D-*glycero*-D-*galacto*-heptose from the avocado marks the first known appearance of an aldoheptose in the plant world.

LaForge[3] discovered D-*manno*-heptulose in the avocado in 1917 and in the same year LaForge and Hudson[4] discovered sedoheptulose (D-*altro*-heptulose) in *Sedum spectabile* Bor. More than forty years elapsed before any additional higher-carbon ketoses were found in nature. In 1959–1960 Charlson and Richtmyer[5] reported the isolation of D-*glycero*-D-*manno*-octulose (I) from both the avocado and from *Sedum* species, and obtained strong evidence for the presence of D-*talo*-heptulose, also, in the avocado.

We have now extracted 95 kg. of the ripe pulp from 400 California avocados (Calavo, Hass variety) with 20% ethanol and, after precipitating the gums with methanol, deionizing, and removing most of the perseitol, D-*manno*-heptulose, D-*erythro*-D-*galacto*-octitol,[5] and *myo*-inositol by crystallization, obtained 304 g. of a residual sirup. This material was chromatographed and the octulose–nonulose fraction rechromatographed several times on columns of cellulose powder by elution with aqueous 1-butanol; some improved techniques that we believe have not been published before are described in the Experimental section. After the faster-moving, dextrorotatory D-*glycero*-D-*manno*-octulose (I) had been separated, a very small amount of a second octulose was obtained that appeared to be homogeneous and was a colorless, hygroscopic, amorphous solid showing $[\alpha]^{20}D$ $-57°$ in water. It was characterized further through its crystalline 2,5-dichlorophenylhydrazone melting at 178–180°. This second octulose from the avocado was proved to be D-*glycero*-L-*galacto*-octulose (II) by the following reactions. First, upon degradation with two molecular equivalents of lead tetraacetate in glacial acetic acid, according to the procedure of Perlin and Brice,[6] it yielded an aldose with the mobility of D-gulose (III) on paper chromatograms, together with a pentose indistinguishable from D-xylose on paper chromatograms and formed probably by further degradation of the D-gulose. Second, upon degradation with oxygen in alkaline solution, according to the procedure of Spengler and Pfannenstiel,[7] it yielded an acid and a lactone with the same mobilities on paper chromatograms as D-*glycero*-L-*galacto*-heptonic acid and its lactone (IV). Third, while the mobilities of the degradation products do not distinguish between enantiomorphs, a comparison of rotations gave a good clue. Hudson[8] called attention to the similarity of physical and chemical properties of the higher-carbon sugars to those of a corresponding hexose that possessed like configuration for the asymmetric carbon atoms 2, 3, 4, and 5. Montgomery and Hudson[9] compared D-*manno*-heptulose and D-mannose similarly. Wolfrom found that comparisons of rotations were valid also when extended to include octuloses[10] and nonuloses.[11] Since the second octulose (II) isolated from the avocado had a molecular rotation ($[M]^{20}D$ $-13,680$) similar to that of L-galactose ($-14,450$), L-*galacto*-heptulose ($-18,000$), and L-*glycero*-L-*galacto*-octulose ($-14,880$[12]), it was assumed to have configuration II rather than that of the enantiomorph of II.

Finally, the D-*glycero*-L-*galacto*-octulose (II) was obtained through synthesis, both enzymatic and chemical. Jones and Sephton[12] earlier had condensed 1,3-dihydroxy-2-propanone phosphate (V) with D-xylose (VI) in the presence of rabbit muscle aldolase and obtained an octulose phosphate from which they prepared 6 mg. of a product that was believed to be, but not positively identified as, D-*glycero*-L-*galacto*-octulose (II). We repeated their enzymatic synthesis, with slight modifications, and obtained a product whose mobilities on paper chromatograms in four different solvent systems were identical to those of the second avocado octulose. These results, together with those described in the next paragraph, give confirmatory proof of the structure of the octulose of Jones and Sephton.

Chemical synthesis of the octulose (II) was achieved by application of Sowden's 2-nitroethanol synthesis, previously used only for the preparation of heptuloses from pentoses.[13] For this, D-gulose (III) was condensed with 2-nitroethanol (VII) in the presence of sodium methoxide. The solid product, isolated as a mixture of

(1) A preliminary account of a part of the work on the octulose was presented before the Division of Carbohydrate Chemistry, 140th National Meeting of the American Chemical Society, Chicago, Ill., September, 1961. Unfortunately, the octulose was reported incorrectly at that time as D-*glycero*-D-*talo*-octulose; Abstracts of papers, p. 1D.

(2) Visiting Scientist of the Public Health Service, September, 1959, to October, 1962.

(3) F. B. LaForge, *J. Biol. Chem.*, **28**, 511 (1917).

(4) F. B. LaForge and C. S. Hudson, *ibid.*, **30**, 61 (1917).

(5) (a) A. J. Charlson and N. K. Richtmyer, *J. Am. Chem. Soc.*, **81**, 1512 (1959); (b) **82**, 3428 (1960).

(6) A. S. Perlin and C. Brice, *Can. J. Chem.*, **34**, 541 (1956).

(7) O. Spengler and A. Pfannenstiel, *Z. Wirtschaftsgruppe Zuckerind.*, **85**, Tech. Tl. 547 (1935).

(8) C. S. Hudson, *Advan. Carbohydrate Chem.*, **1**, 26 (1945); R. M. Hann, A. T. Merrill, and C. S. Hudson, *J. Am. Chem. Soc.*, **66**, 1912 (1944), and earlier papers from their laboratory.

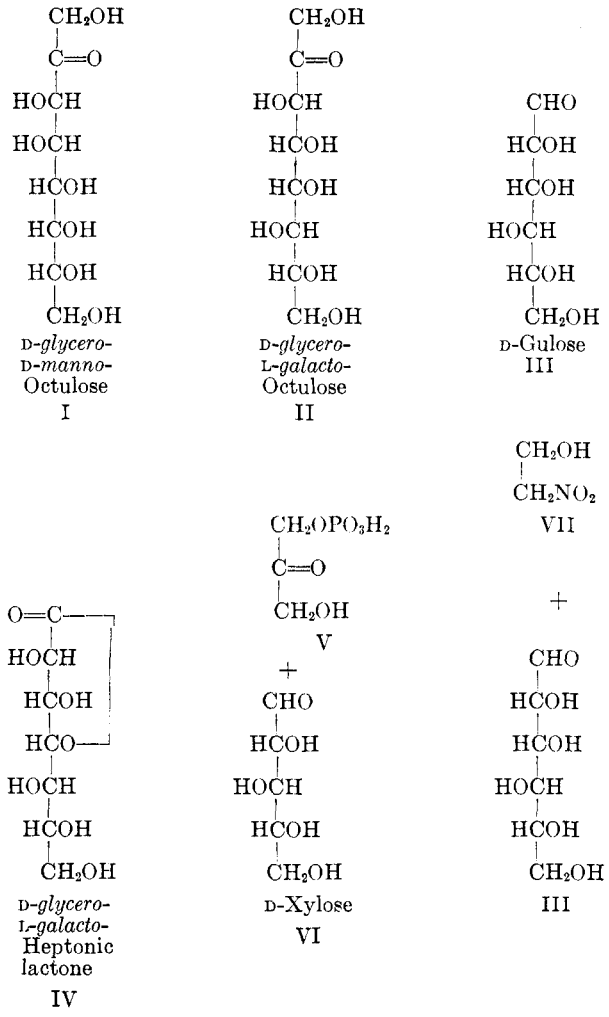
(9) E. M. Montgomery and C. S. Hudson, *ibid.*, **61**, 1654 (1939).

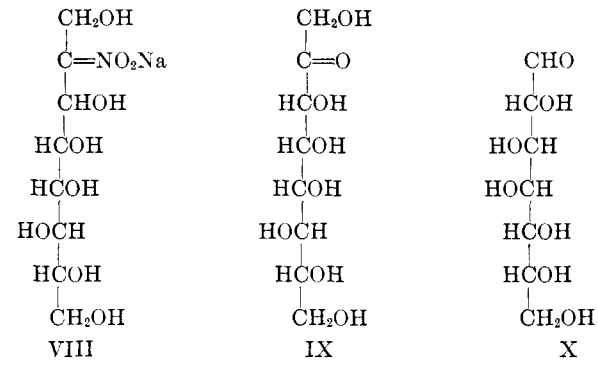
(10) M. L. Wolfrom and P. W. Cooper, *ibid.*, **72**, 1345 (1950).

(11) M. L. Wolfrom and H. B. Wood, Jr., *ibid.*, **77**, 3096 (1955).

(12) J. K. N. Jones and H. H. Sephton, *Can. J. Chem.*, **38**, 753 (1960).

(13) J. C. Sowden, *J. Am. Chem. Soc.*, **72**, 3325 (1950); *Advan. Carbohydrate Chem.*, **6**, 291 (1951); J. C. Sowden and D. R. Strobach, *J. Am. Chem. Soc.*, **80**, 2532 (1958).





sodium salts (VIII), was decomposed with sulfuric acid to give the desired octulose (II) and, presumably, its epimer, D-*glycero*-L-*talo*-octulose (IX).[14] Separation on a cellulose column afforded amorphous D-*glycero*-L-*galacto*-octulose (II), which showed $[\alpha]^{20}D$ −61° in water, a value similar to that (−57°) observed for the second avocado octulose. Paper chromatography and infrared spectroscopy failed to reveal any difference between the synthetic and the natural octuloses and both octuloses afforded the same 2,5-dichlorophenylhydrazone.

Although several aldoheptoses have been reported as constituents of the polysaccharides of Gram-negative bacteria,[15] including D-*glycero*-D-*galacto*-heptose (X) from *Chromobacterium violaceum* (Birch), they have not been found previously as the free sugar, or in either plant or animal sources.[16] We have found, unexpectedly, that D-*glycero*-D-*galacto*-heptose (X) was a heavy contaminant in several of the fractions containing the avocado octuloses and nonuloses.[17] Its presence was first suspected after a portion of one of the octulose fractions was oxidized with two molecular equivalents of lead tetraacetate and D-arabinose was detected; this could not be derived from either avocado octulose, I or II. Later, the D-*glycero*-D-*galacto*-heptose was found to be separable from the second octulose by electrophoresis or by paper chromatography in borate buffers, and finally it was obtained directly from an octulose fraction as its 2,5-dichlorophenylhydrazone and from an octulose–nonulose fraction by direct crystallization. Although D-*glycero*-D-*galacto*-heptose is closely related to D-*manno*-heptulose, we do not believe that our solutions were ever sufficiently alkaline to bring about a Lobry de Bruyn–Alberda van Ekenstein transformation of ketose to aldose. It is conceivable, however, that the heptose may have been a component of an oligosaccharide and was liberated by acid hydrolysis (see Experimental section), but the precise origin of X has not been established.

(14) To be described in a later paper.

(15) See the review by D. A. L. Davies, *Advan. Carbohydrate Chem.*, **15**, 271 (1960).

(16) For a recent review of the higher-carbon sugars, both aldoses and ketoses, natural and synthetic, see J. M. Webber, *ibid.*, **17**, 15 (1962).

(17) In fact, it was the presence of this heptose that was chiefly responsible for our earlier incorrect identification of the second avocado octulose; see ref. 1.

Experimental

Paper chromatography was carried out on Whatman no. 1 filter paper by the descending method at room temperature. The following solvent systems were used: A, ethyl acetate–acetic acid–formic acid–water (18:3:1:4); B, 1-butanol–ethanol–water (40:11:19); C, 1-butanol–pyridine–water (6:4:3); and D, ethyl acetate–pyridine–water saturated with boric acid (12:5:4). Spray reagents used were aniline hydrogen phthalate for aldoses, orcinol-hydrochloric acid for ketoses, alkaline hydroxylamine–ferric chloride for lactones and esters (acids were detected by first converting them into esters by hanging the chromatograms for 10 min. in a tall cylinder containing diazomethane vapors), and silver nitrate (ammoniacal, or in conjunction with sodium hydroxide in ethanol) or sodium metaperiodate–potassium permanganate for alditols, sugars, and other polyhydroxy substances in general. All concentrations were carried out *in vacuo* at temperatures not over 50°; the final drying of sirups was completed in evacuated desiccators over granular calcium chloride. Melting points were determined on a Kofler micro hot stage.

Isolation of Sugars from Avocado Pulp and Chromatographic Separation into Fractions.—Four hundred ripe avocados (Californian Calavo, Hass variety[18]) were freed from skin and seeds, and the pulp (95 kg.) was extracted with 20% ethanol; the extract was freed from gums and deionized as described earlier[5b] and then concentrated until perseitol began to crystallize when the solution was cooled to room temperature. The perseitol weighed 162 g., and on further concentration and cooling the mother liquor deposited two additional crops (the last being slightly contaminated with *myo*-inositol); the total was 332 g. of perseitol. The mother liquor was concentrated to 700 ml., diluted with an equal volume of methanol, and the solution inoculated with seed crystals of perseitol, D-*erythro*-D-*galacto*-octitol,[5] and *myo*-inositol, and cooled slowly to 0°. After removal of the first crop (28 g.), the filtrate was concentrated to a sirup; this was dissolved in 700 ml. of methanol at 45°, and the solution inoculated and cooled to 0°. The second batch (5 g.) was removed, and the filtrate concentrated to a sirup; this was dissolved in 300 ml. of methanol and the solution diluted at 40° with ethanol to incipient cloudiness, inoculated, and cooled slowly to 0°. The total yield of mixed polyhydric alcohols was 42 g. The final filtrate was concentrated to 420 g. of a thick sirup that was dissolved in 300 ml. of methanol at 45° and inoculated with D-*manno*-

(18) Charlson and Richtmyer (ref. 5) used the Fuerte variety of Calavo; the choice of avocado has been dictated only by the variety available in the market at the time the researches were begun.

heptulose. After standing for a week at 0° the D-*manno*-heptulose (109 g.) was filtered and the filtrate concentrated to a dry sirup weighing 304 g.

The residual sirup was dissolved in 200 ml. of methanol and to the solution was added 150 g. of Whatman standard grade cellulose powder that had previously been washed well with hot water and then acetone and dried. The slurry was stirred and 200 ml. of 1-butanol, half saturated with water, added to precipitate the sirupy material onto the cellulose. The methanol was removed on a rotary vacuum evaporator and the free-flowing slurry in 1-butanol transferred to the top of a large cellulose column (100 cm. × 12 cm.); the column had been prepared by packing it under air pressure with an acetone slurry of 2.5 kg. of washed Whatman cellulose powder and the acetone displaced later with half-saturated aqueous 1-butanol. Elution of the column was effected with half-saturated aqueous 1-butanol gradually increasing to fully saturated aqueous 1-butanol. With an automatic collector, 52 fractions, each containing 2.2 l., were obtained and combined later according to their contents as assayed by paper chromatography. Fractions 1–24 contained lower monosaccharides and D-*manno*-heptulose totaling 228 g. Fractions 25–27 consisted principally of D-*glycero*-D-*manno*-octulose[5] (I, 11.2 g.). Fractions 28–37 (total 18.3 g.) appeared to contain the same octulose (I), a second octulose, and also two nonuloses; a partial examination of these fractions will be described later. Fractions 38–52 (36 g.) contained mostly oligosaccharides composed of both aldoses and ketoses, and these fractions, together with fractions 1–27, were saved for further examination at a later date.

Fractions 28–30 and 31–37, being already partially resolved into octulose and nonulose components, respectively, were dissolved separately in small amounts of methanol and additional amounts of polyhydric alcohols (0.5 and 2.6 g., respectively) isolated by slow concentration and crystallization of the solutions at 0°. The filtrates were concentrated to sirups whose paper chromatographic examination showed the presence of oligosaccharides. Accordingly, these components were hydrolyzed by dissolving the sirups each in 5 ml of 0.2 *N* aqueous hydrochloric acid and heating 12 hr. at 95°. The hydrolyzates were deacidified with Duolite A-4 ion-exchange resin. Examination of these hydrolyzates by paper chromatography indicated that the liberated sugars were principally *manno*-heptulose and xylose, with lesser amounts of fructose, glucose, and arabinose.

To maintain the partial separation of octuloses and nonuloses already achieved and to effect their further separation and purification, a cellulose column (85 cm. × 5 cm.) was prepared; on top of this were placed the combined, hydrolyzed fractions 28–30 that had been precipitated onto cellulose and slurried in half-saturated aqueous 1-butanol as described above; the cellulose in the slurry was allowed to settle in a horizontal layer and the solvent was drained to the level of that layer; and finally fractions 31–37 were deposited similarly in a layer on top of fractions 28–30. Elution of this column with half-saturated aqueous 1-butanol removed the lower monosaccharides (2.7 g.), D-*manno*-heptulose (3.3 g.), D-*glycero*-D-*manno*-octulose (2.3 g.), then five fractions (total 4.4 g.) of partially separated octuloses and nonuloses, and finally 2.1 g. of unhydrolyzed oligosaccharides plus some of the second nonulose.

The five fractions that contained most of the remaining octuloses and nonuloses were concentrated to dryness and an additional 0.3 g. of polyhydric alcohols was removed by dissolving the residues in small amounts of methanol and filtering. The five filtrates were precipitated separately onto cellulose powder and the slurries deposited on the top of a cellulose column (100 cm. × 3 cm.) in layers in the same order in which they had been eluted from the previous column. Elution with quarter-saturated aqueous 1-butanol increasing to half-saturated aqueous 1-butanol yielded seven fractions. Fraction A (0.49 g.) was chromatographically pure D-*glycero*-D-*manno*-octulose (I) with $[\alpha]^{20}D$ +26.5° in methanol (*c* 5; previously reported[5] +20°). Fraction B (0.50 g.) was a mixture of the two octuloses (I and II) and a heptose. Fraction C (0.32 g.) contained the second octulose (II) and a heptose (X) separable from it by electrophoresis and by chromatography in solvent D but not in solvents A, B, or C. Fraction D (0.49 g.) contained the second octulose (II), the first nonulose, and some heptose (X). Fraction E (1.08 g.) contained principally the first nonulose, fraction G (0.42 g.) principally the second nonulose, and fraction F (0.50 g.) a mixture of the two nonuloses.

Isolation of D-*glycero*-L-*galacto*-Octulose (II) from Fraction B.—The 0.5 g. of fraction B was dissolved in 100 ml. of water and the heptose contained therein destroyed by adding 0.5 g. of barium carbonate and 0.5 ml. of bromine and stirring vigorously for 10 min. at room temperature. The excess of bromine was removed by aeration, the solids were removed by filtration, and the filtrate was deionized by passage through Dowex 50 and Duolite A-4 ion-exchange resins. Concentration left 0.27 g. of a dry sirup. This residue was dissolved in 10 ml. of methanol, precipitated onto cellulose powder, and the slurry put on top of a cellulose column (100 cm. × 2.5 cm.) in the manner described earlier in this publication. Elution with quarter- to half-saturated aqueous 1-butanol afforded one fraction that appeared to be homogeneous and to contain only the second octulose (D-*glycero*-L-*galacto*-octulose, II) when examined by paper chromatography in all four solvent systems. After filtration through activated carbon (Darco X) the solution was concentrated to a colorless, amorphous, hygroscopic solid that weighed 57 mg. and showed $[\alpha]^{20}D$ −57° in water (*c* 2).

A 19-mg. portion of the second octulose (II) was heated with twice its weight of 2,5-dichlorophenylhydrazine in 1 ml. of methanol on the steam-bath until near dryness. Additional methanol was added and the heating repeated until a total of 5 ml. of methanol had been used and the total heating time had been 45 min. Upon cooling to room temperature the residue crystallized in part; it was washed with ethyl ether seven times by decantation to remove the excess of reagent and the residue was recrystallized from methanol. The **D-*glycero*-L-*galacto*-octulose 2,5-dichlorophenylhydrazone** separated into aggregates of nearly colorless, small needles that melted at 178–180°. The recrystallized first crop weighed only 1.6 mg.; two additional crops (6.5 mg.) were obtained by reheating the original mother liquor with the ethyl ether extract containing the excess of reagent from the first crop of crystals.

Anal. Calcd. for $C_{14}H_{20}Cl_2N_2O_7$: Cl, 17.8. Found: Cl, 18.1.

Degradation of D-*glycero*-L-*galacto*-Octulose (II) to D-Gulose (III) and D-Xylose (VI) with Lead Tetraacetate.—To a solution of 10 mg. of the second octulose (II) in 10 ml. of glacial acetic acid was added 2 ml. of 0.04 *M* lead tetraacetate in glacial acetic acid (2 molecular equivalents). After 15 min. at room temperature the lead was precipitated as the oxalate by the addition of a slight excess of a 10% solution of oxalic acid in acetic acid. The precipitate was removed by centrifugation and the acetic acid evaporated by a current of air. The residue was heated in 10 ml. of 5% aqueous acetic acid for 8 hr. on the steam bath to hydrolyze formyl and glycolyl groups, and the solution was then deacidified with Duolite A-4 ion-exchange resin and concentrated. The product, on paper chromatographic examination in solvents A, B, and C, showed aldose spots corresponding to gulose and xylose only.

Degradation of D-*glycero*-L-*galacto*-Octulose (II) to D-*glycero*-L-*galacto*-Heptonic Acid with Oxygen in Alkaline Solution.—A 2-mg. portion of the second octulose was deposited on the inside surface of a 25-ml. flask by concentrating its solution in 1 ml. of methanol on a rotary evaporator. By means of a three-way stopcock, the flask was evacuated and filled with oxygen from a balloon attached to one branch of the stopcock; through another branch, 0.5 ml. of *N* aqueous potassium hydroxide was introduced; and the flask, with oxygen balloon attached, was rotated overnight. The solution was diluted with water, decationized with Dowex 50 resin, and concentrated. Paper chromatographic examination in solvents A, B, and C showed that the product contained an acid and a lactone with the same mobilities as D-*glycero*-L-*galacto*-heptonic acid and lactone (IV). The lactone was readily distinguishable from D-*glycero*-L-*talo*-heptonic lactone on paper chromatograms.

Enzymatic Synthesis of D-*glycero*-L-*galacto*-Octulose (II) from D-Xylose (VI).—The Jones and Sephton[12] procedure was modified as follows. A mixture of 0.1 g. of D-fructose 1,6-diphosphate tri(cyclohexylammonium) salt, 0.05 g. of D-xylose, and 20 mg. of a commercial muscle aldolase in 9 ml. of water at pH 6.7 was kept at room temperature for 3 days. The proteins were coagulated by heat and filtered; the filtrate was cooled and adjusted to pH 5; and 30 mg. of acid phosphatase was added to hydrolyze the sugar phosphates. The solution was then deproteinized, deionized, and concentrated. Paper chromatographic examination of the product showed, besides fructose and xylose, an octulose with the same mobility in all four solvent systems as the second octulose (II) from the avocado.

The 2-Nitroethanol Synthesis of D-*glycero*-L-*galacto*-Octulose (II) from D-Gulose (III).—A solution of D-gulose was prepared by deionizing 20 g. of α-D-gulose·$CaCl_2$·H_2O with Dowex 50 and

Duolite A-4 resins, and concentrated to a dry sirup. To a solution of this sirup in 20 ml. of methanol was added 10.5 g. of 2-nitroethanol (VII, freshly distilled from a commercial product) in 15 ml. of methanol followed by a solution of sodium methoxide made by dissolving 2.6 g. of sodium in 60 ml. of methanol. The mixture was stirred vigorously at room temperature for 6 hr., then cooled to 0°, and the precipitated sodium salts were filtered and washed in succession with cold methanol, ethyl ether, and petroleum ether (b.p. 90–100°). While still moist, the filter cake was dissolved in ice-cold water and decationized without delay by passage through a column of Dowex 50 resin. Concentration of the effluent yielded 11.6 g. of a dry sirup containing the 2-deoxy-2-nitrooctitols but attempts to crystalline them from methanol and ethanol were unsuccessful. The sirup, therefore, was dissolved in a cold solution containing 1.7 g. of sodium hydroxide in 35 ml. of water and the solution added dropwise, with stirring, to 30 ml. of cold aqueous 50% sulfuric acid. The solution was warmed to 30°, deionized with Dowex 50 and Duolite A-4 resins, and concentrated to a sirup. Paper chromatography indicated that the sirup contained the two expected octuloses and two other orcinol-positive compounds (possibly anhydrooctuloses) in addition to a small amount of D-gulose. These components were separated on a cellulose column (100 cm. × 4 cm.) by elution with quarter- to half-saturated aqueous 1-butanol. The sirupy D-*glycero*-L-*galacto*-octulose fraction (1.36 g., 8.8% over all from the D-gulose calcium chloride compound) showed $[\alpha]^{20}D$ −61° in water (*c* 12) as compared with the value $[\alpha]^{20}D$ −57° observed for the second avocado octulose (II).[19] The infrared spectrum (dried film from methanol) of the chemically synthesized octulose was almost identical with that of the second octulose (II) from the avocado, and paper chromatography in all four solvent systems indicated the identity of the chemically synthesized octulose, the enzymatically synthesized octulose, and the second octulose from the avocado.

The **2,5-dichlorophenylhydrazone** of this synthetic D-*glycero*-L-*galacto*-octulose was prepared as described for the second avocado octulose, but in better yield (59 mg. from 50 mg. of octulose sirup). The twice-recrystallized product melted at 179–181° alone and when mixed with the previously described compound. The infrared spectra of the two products in Nujol mulls were almost identical.

Anal. Calcd. for $C_{14}H_{20}Cl_2N_2O_7$: C, 42.12; H, 5.05; Cl, 17.76; N, 7.02. Found: C, 42.11; H, 5.43; Cl, 17.57; N, 6.93.

Lead Tetraacetate Oxidation of Fraction C to D-Arabinose. Isolation of D-*glycero*-D-*galacto*-Heptose (X) from Fraction C.—A 13-mg. portion of fraction C was oxidized with two molecular equivalents of lead tetraacetate in the manner described earlier in this paper for D-*glycero*-L-*galacto*-octulose (II). The 9 mg. of resulting sirup showed $[\alpha]^{20}D$ −72° in water (*c* 3). On paper chromatograms developed with solvents A, B, and C the main component of the sirup was found to be a pentose with the same mobility as arabinose but readily separable from lyxose, ribose, and xylose. A 3.2-mg. portion of this sirup and 3.5 mg. of 1,1-diphenylhydrazine in 1 ml. of ethanol, kept in the dark at room temperature for 3 days, deposited crystals that were identified as D-arabinose 1,1-diphenylhydrazone by its m.p. of 197–199° and m.m.p. of 198–201° with authentic material prepared similarly.

Another portion (25 mg.) of fraction C in 0.05 ml. of water was applied in a 28-cm. streak to a sheet of S & S no. 589 electrophoresis paper moistened with sodium borate buffer at pH 10. Separation of the heptose and octulose was obtained by applying a 900-v. potential (38–55 ma.) for 3.5 hr. at −5°. The positions of the sugars were determined by spraying test strips with 0.06% sodium metaperiodate in 10% aqueous acetic acid (10 min.) followed by 2% *p*-anisidine in 2% aqueous acetic acid. The slower-moving component was recovered by extraction with methanol, removal of ions and boric acid in the usual manner, and concentration to 10 mg. of dry sirup. The product, crystallized readily from methanol, weighed 4 mg. and was identified as D-*glycero*-β-D-*galacto*-heptose by m.p. 139–141° and m.m.p. 140–141° with an authentic sample of that sugar recrystallized from methanol.

Another portion (27 mg.) of fraction C was refluxed for 2 hr. with 40 mg. of 2,5-dichlorophenylhydrazine in 10 ml. of methanol and then evaporated carefully to dryness on a steam bath. The residue solidified on cooling and was freed from excess of reagent by washing by decantation several times with ether. The D-*glycero*-D-*galacto*-**heptose 2,5-dichlorophenylhydrazone**, on recrystallization from methanol, separated in clusters of small needles (18.4 mg.), m.p. 203–204°. The 2,5-dichlorophenylhydrazone prepared in the same way from an authentic specimen of the heptose also melted at 203–204°, and a mixture melting point was not depressed. The infrared spectra (Nujol mulls) of the two derivatives were identical.

Anal. Calcd. for $C_{13}H_{18}Cl_2N_2O_6$: C, 42.29; H, 4.91; Cl, 19.2; N, 7.6. Found (derivative of compound from avocado): C, 42.19; H, 5.23; Cl, 19.2; N, 7.6.

Direct Crystallization of D-*glycero*-β-D-*galacto*-Heptose from Fraction D.—The 0.49 g. of fraction D, containing D-*glycero*-D-*galacto*-heptose and also some of the second octulose (II) and first nonulose, was dissolved in 1 ml. of methanol and nucleated with a tiny crystal of authentic D-*glycero*-β-D-*galacto*-heptose. During the course of several weeks at room temperature a mass of prismatic crystals formed. The product, filtered and washed with methanol and air dried, weighed 58 mg., melted at 139–140° alone and at 140–141° when mixed with authentic heptose. It mutarotated from $[\alpha]^{20}D$ +47° (5 min.) to +64° (7 hr., constant) in water (*c* 0.5). Montgomery and Hudson[20] reported m.p. 145° and $[\alpha]^{20}D$ +43.9° (4.8 min.) to +69.1° (equilibrium) for anhydrous D-*glycero*-β-D-*galacto*-heptose.

Anal. Calcd. for $C_7H_{14}O_7$: C, 40.00; H, 6.71. Found: C, 40.19, 40.16; H, 7.07, 6.94.

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(19) The values $[\alpha]^{23}D$ −43.4 → −13.4° reported by Jones and Sephton (ref. 12) refer to a very small and obviously rather impure sample of enzymatically synthesized D-*glycero*-L-*galacto*-octulose.

(20) E. M. Montgomery and C. S. Hudson, *J. Am. Chem. Soc.*, **64**, 247 (1942).