CRYSTALLINE D-*glycero*-L-*gluco*-OCTULOSE, CRYSTALLINE METHYL D-*glycero*-α-L-*gluco*-OCTULOPYRANOSIDE, AND SOME RELATED COMPOUNDS

NELSON K. RICHTMYER

National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland 20014 (U. S. A.)

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ABSTRACT

Several compounds in the D-*threo*-L-*ido*-octose series are described. Syrupy D-*threo*-L-*ido*-octose was found to be converted, by long standing, into D-*glycero*-L-*gluco*-octulose (**3**). The same octulose has also been obtained by oxidation of D-*threo*-L-*gulo*-octitol with *Acetobacter suboxydans*. Treatment of D-*glycero*-L-*gluco*-octulose with methanol and an acid catalyst produced methyl D-*glycero*-α-L-*gluco*-octulopyranoside, whose structure was proved by degradation to methyl α-L-*gluco*-heptulopyranoside, the enantiomorph of methyl α-D-*gluco*-heptulopyranoside, whose pyranoside structure was proved in 1942. Some other compounds derived from octulose **3** are also described.

INTRODUCTION

In 1944, Hann, Merrill, and Hudson[1] described the addition of hydrogen cyanide to D-*glycero*-L-*gluco*-heptose (**1**) and the separation of the resulting D-*threo*-L-*gulo*- and D-*threo*-L-*ido*-octonic acids through their crystalline phenylhydrazides. They also described a number of other compounds in the D-*threo*-L-*gulo*-octose series.

RESULTS AND DISCUSSION

The present paper first describes some subsequent studies by Dr. Raymond M. Hann* and Dr. Alice T. Merrill** in the D-*threo*-L-*ido*-octose series, as recorded in their notebooks. By routine procedures, they prepared D-*threo*-L-*ido*-octono-1,4-lactone and the amide and benzimidazole derived from it. They reduced the lactone with sodium amalgam to syrupy D-*threo*-L-*ido*-octose (**2**), and, by further reduction with hydrogen and Raney nickel, converted the octose into the crystalline D-*threo*-L-*ido*-octitol; they acetylated the octitol to give its octaacetate.

A few years ago, several Pyrex beakers remaining from that research and labeled D-*threo*-L-*ido*-octose were observed to contain a crystalline substance. Paper-chromatographic examination of the mixture revealed the probable presence of an

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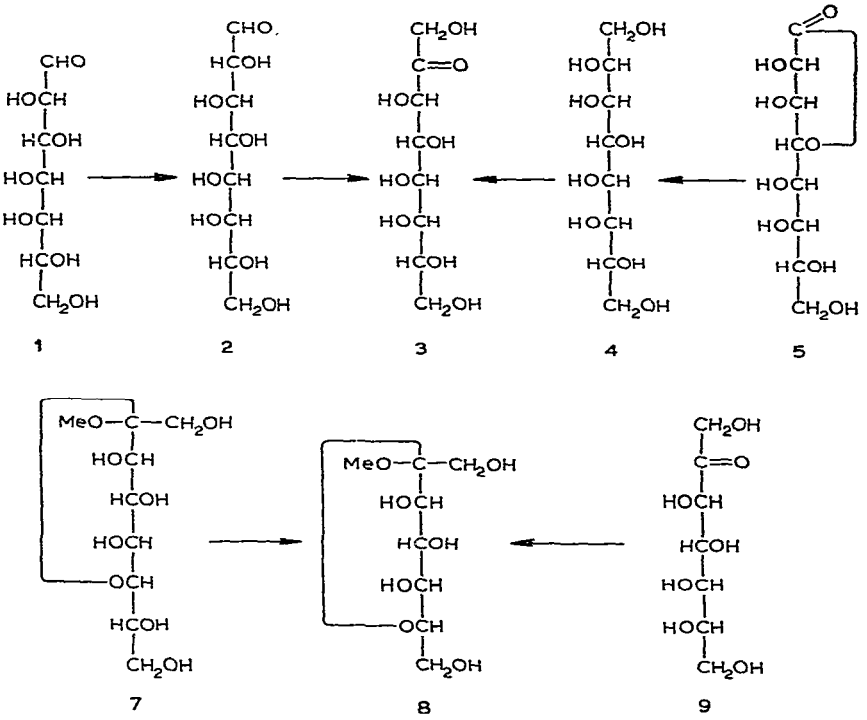
octulose, because of its characteristic color reaction when the chromatogram was sprayed with an orcinol–hydrochloric acid reagent and heated. The octulose was readily isolated in crystalline form by chromatography on a column of cellulose powder. It gave the same phenylosazone as given by D-*threo*-L-*gulo*-octose and the syrupy D-*threo*-L-*ido*-octose (**2**), and could, therefore, only be the related octulose, namely, D-*glycero*-L-*gluco*-octulose (**3**). The same octulose was then prepared by the *Acetobacter suboxydans* oxidation of D-*threo*-L-*gulo*-octitol (≡ D-*erythro*-L-*galacto*-octitol, **4**)[1], which in turn had been obtained by borohydride reduction of D-*threo*-L-*gulo*-octono-1,4-lactone (**5**). This biochemical oxidation of compound **4** again confirms the specificity rule of Bertrand[2] for the action of *Acetobacter xylinum* on polyhydric alcohols as extended by Hann, Tilden, and Hudson[3] to the similar action of *A. suboxydans*. D-*glycero*-L-*gluco*-Octulose (**3**) melts at 166–167° and has $[\alpha]_D^{20}$ −65.6° in water; no mutarotation was observed. This octulose had been prepared earlier by Wolfrom and Cooper[4] as a syrup ($[\alpha]_D^{26}$ −45° in water) by the diazomethane synthesis from D-*glycero*-L-*gluco*-heptonic acid, and later by Jones and Sephton[5] as a syrup ($[\alpha]_D^{27}$ −46.9° in water) by the condensation of 1,3-dihydroxy-2-propanone phosphate with D-lyxose in the presence of rabbit muscle aldolase, followed by hydrolysis of the resulting octulose phosphate with an acid phosphatase.

Compound **3** is the second crystalline octulose to be isolated in this laboratory following the spontaneous isomerization of an octose, presumably through the influence of alkali from the Pyrex container, over the course of many years; the first was D-*glycero*-D-*gulo*-octulose[6] from D-*erythro*-L-*talo*-octose. The only other crystalline octulose known to this author is D-*gluco*-L-*glycero*-3-octulose, which was obtained by Schaffer and Cohen[7] through the dimolecular condensation of D-erythrose under the influence of aqueous calcium hydroxide.

When D-*glycero*-L-*gluco*-octulose (**3**) was boiled (or even kept at room temperature) with methanol and Amberlite IR-120 (H^+) ion-exchange resin, it gave about a 50% yield of a crystalline compound melting at 183–184° and having $[\alpha]_D^{20}$ −96.2° in water. Its structure was proved to be that of methyl D-*glycero*-α-L-*gluco*-octulopyranoside (**7**), because it could be converted in good yield into methyl α-L-*gluco*-heptulopyranoside (**8**) in two steps: (*a*) oxidation with one molecular equivalent of periodate to cleave the molecule between C-7 and C-8 with the formation of an aldehyde group at C-7, and (*b*) borohydride reduction at C-7 to yield compound **8**. Compound **8** was also prepared directly from L-*gluco*-heptulose (**9**), and the two heptulosides were found to be identical through comparison of their pentaacetates; although methyl α-D-*gluco*-heptuloside[8] is known in crystalline form, its enantiomorph (**8**) failed to crystallize. The α-L configuration of methyl α-L-*gluco*-heptulopyranoside (**8**) follows from its high levorotation, and the presence of the pyranoside ring was established by the work of Khouvine and Arragon[9] who oxidized the D form with periodic acid and found that it consumed two moles of reagent and liberated one mole of formic acid per mole of compound.

Some other derivatives of D-*glycero*-L-*gluco*-octulose are also described in the Experimental section.



EXPERIMENTAL

The descriptions of the preparation and properties of the first six compounds were obtained from the notebooks of Dr. Raymond M. Hann and Dr. Alice T. Merrill, formerly of this laboratory. That work was performed in 1944.

D-threo-L-ido-*Octono-1,4-lactone.* — A solution of 40.0 g (0.11 mole) of D-*threo*-L-*ido*-octonic phenylhydrazide hydrate[1] and 41.1 g (0.16 mole) of copper(II) sulfate pentahydrate in 1 liter of water was boiled for 5 h under a reflux condenser, cooled, and filtered. Copper ions were removed by bubbling hydrogen sulfide into the solution and filtering off the copper sulfide; the excess of hydrogen sulfide was removed by aeration. Sulfuric acid was removed by the old method of "balancing out" with aqueous barium hydroxide. The filtrate from the final barium sulfate precipitate was first concentrated *in vacuo* to a small volume and then evaporated in a glass dish on a steam bath to crystallization. The dish and its contents were finally dried in an oven at 55° until the weight became constant; yield 22.3 g (79%). The product was recrystallized from 95% ethyl alcohol (20 ml/g) to give 18.6 g (66%) of small prisms of pure D-*threo*-L-*ido*-octono-1,4-lactone having m.p. 163–164°. Aqueous solutions of this lactone mutarotate (*c* 1, water), the preparation described showing $[\alpha]_D^{20}$ +62.0 (3 min) → +59.9° (6 min, constant); another recrystallized sample of m.p. 163–164° was recorded by Dr. Merrill as showing $[\alpha]_D^{20}$ +70.5 (5 min) → +59.3° (5.5 h, constant overnight). The lactone is soluble in hot methanol, ethyl alcohol, pyridine, or acetic acid, and almost insoluble in acetone or ethyl acetate.

Anal. Calc. for $C_8H_{14}O_8$: C, 40.34; H, 5.92; mol. wt., 238. Found: C, 40.23; H, 5.93; mol. wt. (by titration with sodium hydroxide), 241.

This D-*threo*-L-*ido*-octonolactone is, presumably, a γ-lactone, because its relatively strong dextrorotation is in accord with Hudson's lactone rule[10]. Its infrared spectrum (Nujol mull), however, shows a carbonyl absorption band at 1743 cm^{-1}; this value is in the 1726–1760 cm^{-1} range considered to be characteristic of δ-lactones of aldonic acids, and not in the 1765–1790 cm^{-1} range considered to be characteristic of γ-lactones[11]. Both the analysis and the measurement of optical rotation have recently been repeated and found to be in accord with the earlier data. A sample of the lactone in 2-methoxyethanol was boiled for 6 h, and recovered with m.p. and i.r. spectrum unchanged. For comparison, the i.r. spectrum of D-*glycero*-D-*ido*-heptono-1,4-lactone {$[\alpha]_D^{20}$ $-77 \rightarrow -67°$ (ref. 12), and thus in accord with Hudson's rule} showed its carbonyl absorption band at 1765 cm^{-1}.

D-threo-L-ido-*Octonamide.* — A solution of 2.0 g of the foregoing octonolactone in 20 ml of liquid ammonia was allowed to evaporate at room temperature. The crystalline residue was dried further, in an evacuated desiccator containing calcium chloride and concentrated sulfuric acid, until the odor of ammonia could no longer be detected. The product was dissolved in 15 ml of water, and the solution was filtered through a layer of decolorizing carbon, diluted with 20 ml of ethyl alcohol, and nucleated with crystals obtained earlier. The amide was allowed to crystallize for several hours in a refrigerator; the crystals were then filtered off, washed with 50% ethyl alcohol, and dried in a desiccator. The clusters of small prisms weighed 1.2 g (56%) and, after a second, similar recrystallization, had m.p. 177–178° (dec.) and $[\alpha]_D^{20}$ +26.7° (*c* 0.9, water).

Anal. Calc. for $C_8H_{17}NO_8$: C, 37.64; H, 6.72; N, 5.49. Found: C, 37.57; H, 6.83; N, 5.46.

2-(D-threo-L-ido-*1,2,3,4,5,6,7-Heptahydroxyheptyl)benzimidazole.* — A mixture of 2.0 g (8.4 mmoles) of the octonolactone, 1.2 g (11.1 mmoles, 1.2 mol. equiv.) of *o*-phenylenediamine, 4 ml of water, 2 ml of ethyl alcohol, 1.6 ml of concentrated hydrochloric acid, and 0.8 ml of 85% orthophosphoric acid was warmed until dissolution was complete, and the solution was heated for 2 h at 135°. The mixture was cooled and dissolved in 10 ml of water. The solution was filtered through a mat of decolorizing carbon, diluted to 30 ml with water, and made neutral with ammonium hydroxide. Because no precipitation occurred, even on cooling to 5°, the solution was evaporated *in vacuo*, and the residue was extracted with three portions (total, 20 ml) of boiling ethyl alcohol. The extract was filtered, concentrated to 10 ml, and kept overnight; a small amount of ammonium chloride that separated was removed by filtration. On standing for several days in a refrigerator, the filtrate deposited 0.9 g (33%) of the substituted benzimidazole. It was recrystallized from 27 ml of ethyl alcohol, from which it separated as clusters of needles having m.p. 194–195° and $[\alpha]_D^{20}$ +24.4° (*c* 0.8, M hydrochloric acid). The sign of rotation is in accord with that expected from the benzimidazole rule[13]. The magnitude of the molecular rotation ($[M]_D^{20}$ +8000°) is very close to that of the benzimidazole derived from D-*glycero*-D-

ido-heptonic acid ($[M]_D^{20}$ −8200°)[14] but, as expected, the signs are opposite. The rotation ($[M]_D^{20}$ −5200°)[14] of the benzimidazole derived from D-idonic acid is somewhat lower.

Anal. Calc. for $C_{14}H_{20}N_2O_7$: C, 51.22; H, 6.14; N, 8.53. Found: C, 51.23; H, 6.01; N, 8.51.

Syrupy D-threo-L-ido-*octose* (**2**). — A total of 50 g of the octonolactone was reduced, in three portions, with a total of 6 kg of 2.5% sodium amalgam in the usual way. The sodium sulfate was removed by precipitation with ethyl alcohol and filtration, and the filtrate was evaporated to a syrupy residue of octose that was subjected to various treatments with solvents in unsuccessful attempts to obtain the sugar crystalline. An amount of several grams of D-*threo*-L-*ido*-octitol was isolated, and this was identified with the same octitol prepared as described next. A portion of the syrupy octose was converted into the phenylosazone, which was identical (by m.p., mixed m.p., and i.r. spectrum) with D-*glycero*-L-*gluco*-octulose phenylosazone prepared from the epimeric D-*threo*-L-*gulo*-octose[1]. A crystalline phenylhydrazone could not be obtained.

D-threo-L-ido-*Octitol* (≡ L-threo-L-galacto-*octitol*). — A solution of syrupy D-*threo*-L-*ido*-octose (5 g) in water (75 ml) was reduced with Raney nickel (3 g) and hydrogen under a pressure of 1,000 lb. in.$^{-2}$ for 6 h at 100°. After the mixture in the bomb had cooled to room temperature overnight, it was filtered, and the filtrate was evaporated *in vacuo* to a scaly, moss-like material. This material, when dissolved in 5 ml of boiling water and diluted with 5 ml of methanol, crystallized at once. The clusters of small plates had m.p. 163–164° and weighed 2.6 g. After two additional recrystallizations from water–methanol, it had m.p. 165–166° and $[\alpha]_D^{20}$ +0.75° (*c* 2, water).

Anal. Calc. for $C_8H_{18}O_8$: C, 39.67; H, 7.49. Found: C, 39.63; H, 7.46.

D-threo-L-ido-*Octitol octaacetate*. — Acetylation of 1.5 g of the octitol was accomplished by refluxing it with acetic anhydride (15 ml) and pyridine (15 ml) for 5 min. Cooled and poured into water, the mixture yielded 3.1 g (86%) of crystalline octitol octaacetate. Three recrystallizations from ethyl alcohol (4 ml/g) gave 2.3 g of small prisms having m.p. 123–124° and $[\alpha]_D^{20}$ −18.6° (*c* 1, chloroform).

Anal. Calc. for $C_{24}H_{34}O_{16}$: C, 49.83; H, 5.92; CH_3CO, 59.5. Found: C, 49.77; H, 5.91; CH_3CO, 59.4.

D-glycero-L-gluco-*Octulose* (**3**). — Three small, Pyrex beakers that had originally contained syrupy D-*threo*-L-*ido*-octose (**2**) prepared in 1944 by Dr. Raymond M. Hann and Dr. Alice T. Merrill were recently observed to contain considerable crystalline material. Paper-chromatographic examinations indicated the presence of much aldose (octose) and octulose, together with a small proportion of a nonreducing substance (octitol). The mixture (15 g) was dissolved in water, and the solution was freed of any ionizable substances by passage through Amberlite IR-120 and Duolite A-4 ion-exchange resins. The eluate was evaporated to a syrup that was made into a smooth slurry with 10.5 g of dry cellulose powder and quarter-saturated aqueous butyl alcohol; this slurry was put on top of a cellulose column (effective size 95 × 4 cm)

as described earlier[15]. Elution was begun with quarter-saturated, aqueous butyl alcohol. A forerun of 2.5 liters was collected, and then fractions (15 ml each) were obtained with an automatic fraction-collector. After collection of the forerun, a type of gradient elution was used, so that, by the time a total of 5 liters had been collected, the eluant was approximately half-saturated, aqueous butyl alcohol. Paper chromatography of the forerun and of fractions 1–260 showed nothing of interest. Fractions 261–400 showed, on paper chromatograms, strong evidence that an aldose (octose) was contained therein, and fractions 421–840, after concentration, yielded the crystalline octulose, which had first crystallized spontaneously in combined fractions 681–700. Fractions 841–1460 yielded 0.8 g of crystals quite different in appearance from that of the octulose and, after recrystallization from methanol, the 0.6 g of clusters of prisms was identified, through m.p. and mixed m.p. of 165–166°, as well as through paper chromatography, as the D-*threo*-L-*ido*-octitol already described.

The D-*glycero*-L-*gluco*-octulose (**3**) isolated from fractions 421–840 weighed 5.7 g. After two recrystallizations from methyl alcohol, the resulting stout prisms melted at 166–167° and had $[\alpha]_D^{20}$ −65.6 ±0.7° (*c* 1.4, water); no mutarotation (between 5 min and 24 h) could be detected.

Anal. Calc. for $C_8H_{16}O_8$: C, 40.00; H, 6.71. Found: C, 39.92; H, 6.55.

A portion (0.19 g) of octulose **3** was converted into D-*glycero*-L-*gluco*-octulose phenylosazone by the procedure used earlier for the preparation of the phenylosazone from D-*threo*-L-*gulo*-octose[1]. The yield was only 23%. The m.p. was 203–204° (dec.), and this was not depressed when the compound was mixed with the original phenylosazone[1]. The i.r. spectra of the two specimens were identical.

D-glycero-L-gluco-*Octulose phenylosotriazole.* — A suspension of the phenylosazone (3.47 g) in a solution of copper(II) sulfate pentahydrate (2.29 g; 1.1 molecular equivalents) in water (300 ml) was boiled for 2 h. The mixture was processed as described for preparation of primeverulose phenylosotriazole[16], and yielded 1.57 g (58%) of crystalline octulose phenylosotriazole. After two recrystallizations from ethyl alcohol, the product was obtained as fine needles which had m.p. 170–171° and $[\alpha]_D^{20}$ −43.3° (*c* 2, pyridine). The sign of rotation is in accord with the phenylosotriazole rule of El Khadem[17].

Anal. Calc. for $C_{14}H_{19}N_3O_6$: C, 51.69; H, 5.89; N, 12.92. Found: C, 51.67; H, 5.74; N, 13.04.

D-glycero-L-gluco-*Octulose phenylosotriazole hexaacetate.* — Acetylation of the octulose phenylosotriazole (0.27 g) with acetic anhydride and pyridine for 10 days yielded 0.34 g (71%) of the crystalline hexaacetate. It separated as needles from methanol, and had m.p. 152–153° and $[\alpha]_D^{20}$ −93.4° (*c* 2, chloroform).

Anal. Calc. for $C_{26}H_{31}N_3O_{12}$: C, 54.07; H, 5.41; N, 7.28; CH_3CO, 44.7. Found: C, 54.25; H, 5.16; N, 7.15; CH_3CO, 45.0.

D-glycero-L-gluco-*Octulose phenylosotriazole hexabenzoate.* — Benzoylation of the octulose phenylosotriazole (0.36 g) with benzoyl chloride and pyridine for 3 days at room temperature yielded a syrupy product that contained much benzoic anhydride. The two compounds were readily separated on a column of silica gel by eluting with

benzene until all of the benzoic anhydride had been removed, and then eluting the phenylosotriazole hexabenzoate with 9:1 benzene–ether. The hexabenzoate (0.75 g; 70%), twice recrystallized from methanol, formed clusters of small, elongated prisms having m.p. 79–80° (to a viscous melt) and $[\alpha]_D^{20}$ −82.0° (*c* 2, chloroform).

Anal. Calc. for $C_{56}H_{43}N_3O_{12}$: C, 70.80; H, 4.56; N, 4.42. Found: C, 70.59; H, 4.37; N, 4.38.

D-glycero-L-gluco-*Octulose* (*2,5-dichlorophenyl*)*osazone.* — This (dichlorophenyl)osazone was prepared by heating 0.24 g (1 mmole) of octulose **3** in 10 ml of water with 0.71 g (4 mmoles) of (2,5-dichlorophenyl)hydrazine and 0.23 ml (4 mmoles) of glacial acetic acid for 5 h on a steam bath. The crystalline product was filtered off, and successively washed with 10% aqueous acetic acid, cold ethyl alcohol, and ethyl ether. The yield was only 0.02 g. However, when prepared in the same way from D-*threo*-L-*gulo*-octose[1], the yield was 0.12 g of bright-yellow needles. The i.r. spectra of the two samples were identical. The m.p. was 230° (dec.) and the optical rotation was $[\alpha]_D^{20}$ +95.7 (7 min) → −3.7° (35 days; *c* 1, pyridine).

Anal. Calc. for $C_{20}H_{22}Cl_4N_4O_6$: C, 43.18; H, 3.99; Cl, 25.50; N, 10.07. Found: C, 43.30; H, 3.83; Cl, 25.27; N, 9.88.

D-glycero-L-gluco-*Octulose* (**3**) *from* D-threo-L-gulo-*octitol* (**4**) *by action of* Acetobacter suboxydans. — D-*threo*-L-*gulo*-Octonolactone[1] (**5**) (32 g) was reduced with sodium borohydride (32 g) to give the same D-*threo*-L-*gulo*-octitol (24 g) that Hann, Merrill, and Hudson[1] had obtained by reduction of both D-*threo*-L-*gulo*-octose and D-*erythro*-L-*galacto*-octose with hydrogen and Raney nickel. The octitol (24 g) was then incubated with *Acetobacter suboxydans* (ATCC No. 621) for 12 days at 30° by the same procedure that had been used earlier in this laboratory[18]. Deproteinization, deionization, and crystallization yielded 23.3 g of octulose **3**.

Methyl D-glycero-α-L-gluco-*octulopyranoside* (**7**). — A mixture of 2.0 g of the octulose (finely ground in an agate mortar), a spoonful (4.3 g, dry wt.) of Amberlite IR-120 (H^+) ion-exchange resin that had been thoroughly washed with methanol, and 150 ml of methanol was boiled under a reflux condenser for 16 h. The mixture was cooled and filtered, and the filtrate was stirred with some Duolite A-4 ion-exchange resin to ensure the removal of any acid; the suspension was filtered through a small amount of decolorizing carbon (Darco X), and the filtrate was evaporated *in vacuo*. The resulting syrup crystallized on being dissolved in the minimum volume of methanol and the solution left to evaporate over a week end at room temperature. The product (1.05 g, 50%) was recrystallized from methanol: small prisms having m.p. 183–184° and $[\alpha]_D^{20}$ −96.2° (*c* 2, water).

Anal. Calc. for $C_9H_{18}O_8$: C, 42.52; H, 7.14. Found: C, 42.64; H, 6.95.

Examination of the mother liquor from the crystalline octuloside, on a paper chromatogram developed in 6:4:3 (v/v) butyl alcohol–pyridine–water and sprayed with an orcinol–hydrochloric acid reagent, showed the presence of the octuloside, a very small proportion of octulose, and a small proportion of a compound having a greater mobility than that of the crystalline octuloside. When a mixture of 0.50 g of the octulose, 3.9 g of the Amberlite IR-120 resin, and 175 ml of methanol was stirred

for 30 h and then kept for 2 weeks at room temperature, paper chromatography showed that the octuloside was almost the only constituent, and, indeed, 0.38 g of it was isolated in crystalline form. The ready formation of a methyl ketopyranoside at room temperature was noted earlier by Montgomery and Hudson[19]; they observed that D-*manno*-heptulose was converted by 0.25 M methanolic hydrochloric acid in 3 h at 20° into a compound that was undoubtedly the pyranoside form because it was also the principal product when the solution was heated.

Degradation of methyl D-glycero-α-L-gluco-*octulopyranoside* (7) *to methyl* α-L-gluco-*heptulopyranoside* (8), *and identification of the latter compound as the penta-acetate.* — To a solution of the octuloside (1.00 g) in water (100 ml) was added dropwise a solution of sodium metaperiodate (0.93 g; 1.1 molecular equivalents) in water (40 ml). The next morning, the solution was stirred with a mixture of Amberlite IR-120 and Duolite A-4 ion-exchange resins, the suspension was filtered, and this process was repeated. Next, sodium borohydride (2 g) was added, and the solution was kept over a week end. Sodium ions were removed by stirring the solution twice with fresh batches of Amberlite IR-120 resin, the suspension was filtered, and the filtrate was evaporated to dryness *in vacuo*. Boric acid was removed by dissolving the syrup in methanol, evaporating the solution to dryness, and repeating the operation several times. The resulting syrup weighed 0.80 g, and paper chromatograms showed that it probably contained a large proportion of methyl α-L-*gluco*-heptulopyranoside, together with smaller proportions of the heptulose, octulose, and octuloside.

Because the syrup did not crystallize, it was treated with acetic anhydride (8 ml) and pyridine (10 ml) for several days, and the mixture was then processed in the usual way. The resulting syrup (1.5 g) was dissolved in a very small volume of ethyl alcohol, and the solution was inoculated with a needle crystal of methyl α-L-*gluco*-heptulopyranoside pentaacetate (prepared as described next), and carefully and slowly diluted with a large volume of water. The product, filtered and washed with water, weighed 0.40 g and melted at 75–76°; the m.p. was not depressed when the compound was mixed with authentic material. The mother liquor yielded an additional 0.65 g (total yield 61%, from the octuloside) of the stable, prismatic dimorph having m.p. 109–110°, a value that was not depressed when the compound was mixed with authentic material.

*Methyl-*α-L-gluco-*heptulopyranoside pentaacetate.* — A mixture of 2.25 g of finely powdered L-*gluco*-heptulose[20] (9), 3.9 g of Amberlite IR-120 (H^+) ion-exchange resin that had been well washed with methanol, and 150 ml of methanol was boiled for 18 h under a reflux condenser. The mixture was filtered, the filtrate was stirred with Duolite A-4 resin and Darco X decolorizing carbon, and the suspension was filtered. The colorless filtrate was evaporated *in vacuo* to a syrup (2.40 g, 100%). Paper chromatography showed that the principal constituent had the same mobility as a sample of Austin's methyl α-D-*gluco*-heptulopyranoside[21]; however, there appeared to be small proportions of six other compounds present (as there were, also, in a crude syrup prepared from D-*gluco*-heptulose by the same procedure), and crystallization could not be induced. The material was therefore fractionated on a

cellulose column (100 × 4 cm i.d.) by elution with butyl alcohol half-saturated with water. One fraction (0.75 g) showed only the desired heptuloside on paper chromatograms, but still would not crystallize. After a month, this material was acetylated with acetic anhydride and processed in the usual way. The resulting syrup crystallized readily upon addition of a little ethyl alcohol to it. The remainder of the syrup that had been eluted from the cellulose column was acetylated similarly, and the two batches were combined and crystallized from ethyl alcohol by the addition of water. The first crop of methyl α-L-*gluco*-heptulopyranoside pentaacetate consisted of 2.81 g of needles melting at 76–77°. Austin[21] had reported m.p. 110° for the enantiomorph. Upon recrystallization from ethyl alcohol by careful addition of water, the product again separated as needles, but on standing overnight, the needles were converted into small, chunky prisms. This modification melted at 110°, in agreement with the value found by Austin. The optical rotation of $[\alpha]_D^{20}$ −77.9° (c 2, chloroform) is similar in magnitude to the value $[\alpha]_D^{20-25}$ +78.5 ±0.5° (*c* 2.2, chloroform) reported by Austin for the D-form, but of the opposite sign. The total yield of crystalline material was 3.14 g (71% from the octulose). Incidentally, a sample of the D form prepared for purposes of comparison melted also at 76–77° and, after two recrystallizations, still persisted as the lower-melting modification.

Anal. Calc. for $C_{18}H_{26}O_{12}$: C, 49.77; H, 6.03. Found: (needles) C, 49.67; H, 5.88; (prisms) C, 49.50; H, 5.89.

Negative results. — Although the following compounds were prepared by standard procedures, none of them could be obtained in crystalline form (for the acetates, even after chromatography on silica gel): a heptaacetate and an isopropylidene acetal of octulose **3**, and a hexaacetate and an isopropylidene acetal of the octuloside **7**. D-*glycero*-L-*gluco*-Octulose (2,5-dichlorophenyl)hydrazone could not be prepared.

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