globular clusters of crystals separated. These were recrystallized from ethyl acetate by the addition of petroleum ether; yield 2.5 g., m.p. 223–224°.

Anal. Calcd. for $C_{26}H_{18}O_9$: C, 65.82; H, 3.82. Found: C, 65.88; H, 3.69.

Penta-O-acetyl-*meso*-(*glycero-gulo*)-heptaric Anhydride.—The procedure of Adelman and Breckenridge[17] for acetylating amorphous zinc *meso*-(*glycero-gulo*)-heptarate with acetyl chloride (to produce penta-O-acetyl-*meso*-(*glycero-gulo*)-heptaric acid) was followed and the crude sirup obtained on solvent removal from the dried chloroform extract was maintained for several days in a vacuum desiccator. Crystals formed, in low yield, that were separated from adherent sirup by filtration and were recrystallized from ethyl acetate by the addition of petroleum ether; m.p. 189.5–190°. A solution of the substance in 75% ethanol was initially neutral.

Anal. Calcd. for $C_{17}H_{20}O_{13}$: C, 47.22; H, 4.66. Found: C, 47.04; H, 4.42.

***dialdehydo-meso*-(*glycero-gulo*)-Dialdoheptose Pentaacetate (IX).**—Penta-O-acetyl-*meso*-(*glycero-gulo*)-heptaric acid (VII, 800 mg., m.p. 122°)[17] was stirred at room temperature with an equal weight of phosphorus pentachloride and 25 ml. of acetyl chloride. The filtered reaction mixture was concentrated under reduced pressure to half-volume and again filtered. The residue obtained on complete solvent removal under reduced pressure was crystallized from ether–petroleum ether; yield 310 mg. m.p. 105°. This material, presumably the bis-(acid chloride) VIII, exhibited a strong halogen test with alcoholic silver nitrate but was too unstable for characterization.

An amount of 300 mg. of the above product was immediately reduced in xylene solution with palladium-on-barium sulfate as described above for the preparation of *dialdehydo-galacto*-dialdohexose tetraacetate and the product obtained after a 4-hr. reaction time was isolated in the same manner and was recrystallized from purified ethyl acetate; m.p. 133–134°. The substance exhibited a strong Fehling reduction and was halogen-free.

Anal. Calcd. for $C_{17}H_{22}O_{12}$: C, 48.80; H, 5.30. Found: C, 48.78; H, 5.44.

COLUMBUS 10, OHIO

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

The Preparation of Higher-carbon Sugars from D-Mannose Including Crystalline D-Manno-L-*manno*-octose

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RECEIVED APRIL 8, 1953

D-Manno-L-*manno*-octose has been crystallized for the first time and characterized through its phenylosazone and phenylosotriazole derivatives. D-Manno-L-*manno*- and D-manno-L-*gluco*-octonic acids as well as a number of their derivatives have been prepared. The rotations of the amides, phenylhydrazides and benzimidazoles of both acids conform to their respective rules of rotation. Some compounds in the heptonic series were reinvestigated and new derivatives were prepared. D-Manno-D-*talo*-heptonic amide conforms to the amide rule.

The research described in the present communication is a continuation[3] of the synthesis of higher-carbon sugars and their intermediates from D-mannose. For this purpose barium D-manno-D-*gala*-heptonate described by Montgomery and Hudson[3] was again prepared and from the mother liquors barium D-manno-D-*talo*-heptonate was isolated. Purification of the acid from the latter salt was effected through the phenylhydrazide[4] which in turn was converted[5] to D-manno-D-*talo*-heptonic lactone.[6] From the pure lactone the crystalline cadmium salt and the amide were prepared for the first time. The negative rotation of the amide conformed to the amide rule.[7]

Barium D-manno-D-*gala*-heptonate was converted to the lactone in the customary way[8] and the latter substance reduced to D-manno-D-*gala*-heptose.[9] The physical constants of previously known substances in the heptonic series were verified and the [α]D value, previously recorded for barium D-manno-D-*gala*-heptonate,[3] was corrected. Crystalline cadmium D-manno-D-*gala*-heptonate was also prepared.

When hydrogen cyanide addition to D-manno-D-*gala*-heptose was carried out with sodium cyanide and calcium chloride,[10] the D-manno-L-*manno*-octonic acid was separated from its epimer through its less soluble calcium salt or phenylhydrazide and obtained in pure form as the lactone. The D-manno-L-*gluco*-octonic acid, which had not been isolated previously, was obtained pure either from the partially purified calcium salt or from the phenylhydrazide. Pure derivatives of the two epimeric acids, such as the epimeric phenylhydrazides and benzimidazoles, were prepared from D-manno-L-*manno*-octonic lactone and D-manno-L-*gluco*-octonic acid; the rotations of these derivatives conformed to the phenylhydrazide[11] and benzimidazole[12] rules, respectively. Crystalline sodium D-manno-L-*gluco*-octonate, D-manno-L-*gluco*-octonic amide and ethyl D-manno-L-*manno*-octonate are also described in the present work.

When hydrogen cyanide addition to D-manno-D-*gala*-heptose was carried out with sodium cyanide and ammonium chloride, a crystalline, nitrogen-containing compound was obtained in good yield

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(1) Blockson Chemical Company, Joliet, Illinois.

(2) Deceased: (a) April 30, 1949; (b) December 27, 1952.

(3) Edna M. Montgomery and C. S. Hudson, THIS JOURNAL, **64**, 247 (1942); *cf.* also C. S. Hudson, *Advances in Carbohydrate Chem.*, **1**, 5 (1945).

(4) C. S. Hudson, THIS JOURNAL, **39**, 462 (1917), pointed out that this phenylhydrazide, originally prepared by Peirce (ref. 16), conformed to the phenylhydrazide rule of rotation.

(5) R. M. Hann and C. S. Hudson, *ibid.*, **56**, 957 (1934).

(6) H. S. Isbell, *J. Research Natl. Bur. Standards*, **20**, 97 (1938).

(7) C. S. Hudson, THIS JOURNAL, **40**, 813 (1918).

(8) E. Fischer and F. Passmore, *Ber.*, **23**, 2226 (1890).

(9) The condensation of nitromethane with D-mannose to yield, after hydrolysis of the resulting nitroalcohols, the two epimeric heptoses has been described recently by J. C. Sowden and R. Schaffer [THIS JOURNAL, **73**, 4662 (1951)].

(10) C. S. Hudson, Olive Hartley and C. B. Purves, *ibid.*, **56**, 1248 (1934).

(11) P. A. Levene, *J. Biol. Chem.*, **23**, 145 (1915); C. S. Hudson, ref. 4.

(12) N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **64**, 1612 (1942).

directly from the reaction mixture. Since this substance was found to be ammonium D-manno-L-*manno*-octonate, we wish to emphasize the shift toward the *manno* configuration for carbon atoms 2, 3, 4 and 5 in this mannoöctonic series by use of a more acidic reagent such as "ammonium cyanide" in contrast to the preceding use of "calcium cyanide" which favored the formation of a larger proportion with the *gluco* configuration. Similar findings in the hexonic series have been demonstrated in a practical synthesis of C^{14}-labeled D-glucose and D-mannose by the addition of hydrogen cyanide to D-arabinose.[13]

D-Manno-L-*manno*-octonic lactone was reduced by sodium amalgam to the corresponding octose, D-manno-L-*manno*-octose, which has been obtained in the crystalline condition for the first time. It forms a phenylhydrazone of low solubility and a phenylosazone, both of which have been previously described by Fischer and Passmore,[8] and the octose was further characterized as the phenylosotriazole and phenylosotriazole hexaacetate. Since D-manno-L-*manno*-octose shows mutarotation from +11.7° to −7.5° (Table I) and possesses the configuration of L-mannose for its carbon atoms 2, 3, 4 and 5, it may be regarded as D-manno-β-L-*manno*-octose. The crystalline octose was not fermented by bakers' yeast.

Acknowledgment.—One of us (J.V.K.) held the Chemical Foundation Research Associateship while carrying out this research.

Experimental

Heptonic Series

Barium D-Manno-D-*gala*-heptonate.—The barium salt was prepared from vegetable ivory according to the directions of Montgomery and Hudson.[3] From 2.4 kg. of vegetable ivory there was obtained 1087 g. of crude barium D-manno-D-*gala*-heptonate. This was converted to the lactone by customary procedure and the lactone was readily obtained in pure state (m.p. 149–151°,[14] $[\alpha]^{20}\text{D}$ −74.2° in water, *c* 2) by recrystallization from ethanol. A pure preparation of barium D-manno-D-*gala*-heptonate was made by treating the pure lactone with the theoretical quantity of barium hydroxide. After several recrystallizations from water, it showed $[\alpha]^{20}\text{D}$ +4.2° in water[15] (*c* 1) and after neutralization with twice the theoretical quantity of hydrochloric acid it showed $[\alpha]^{20}\text{D}$ −9.3° (extrapolated value calcd. as the free acid) mutarotating to −60° (constant after 40 hr., calcd. as lactone) (*c* (barium salt) 6.7, *l* 4).

D-Manno-D-*talo*-heptonic Phenylhydrazide.—The mother liquor (10 l.) from the crude barium D-manno-D-*gala*-heptonate was concentrated to 4 l. and 213 g. of crude barium D-manno-D-*talo*-heptonate slowly crystallized from the solution on several months standing near 0°. Further concentration followed by the addition of ethanol precipitated an additional 340 g. of this salt. Recrystallization of the combined salts from 6 parts of water gave 333 g. of crude barium D-manno-D-*talo*-heptonate with $[\alpha]^{20}\text{D}$ +3.8° which could not be purified by further recrystallization. The crude salt was then converted to the impure phenylhydrazide which could be purified, though with considerable loss, by several recrystallizations from 15 parts of 70% ethanol to a constant $[\alpha]^{20}\text{D}$ value of −24.0° in water (*c* 0.7) and m.p. 194–195°. Peirce[16] reported $[\alpha]^{27}\text{D}$ −25.8° and m.p. 190° for this substance.

Anal. Calcd. for $C_{13}H_{20}O_7N_2$: C, 49.36; H, 6.37; N, 8.86. Found: C, 49.14; H, 6.37; N, 8.72.

D-Manno-D-*talo*-heptonic Lactone.—Pure D-manno-D-*talo*-heptonic phenylhydrazide was converted into the crystalline lactone in 86% yield according to the procedure of Hann and Hudson.[5] After recrystallization from ethanol, the lactone showed $[\alpha]^{20}\text{D}$ −34.9° in water (*c* 0.6) and m.p. 131–132°. Isbell[6] recorded $[\alpha]^{20}\text{D}$ −35.7° in water (*c* 4) and m.p. 130° for this substance.

Anal. Calcd. for $C_7H_{12}O_7$: C, 40.38; H, 5.81. Found: C, 40.41; H, 5.87.

Barium D-Manno-D-*talo*-heptonate.—This salt has been prepared by Ettel.[17] Its specific rotation, not hitherto recorded, has now been found to be $[\alpha]^{20}\text{D}$ +1.5° in water (*c* 0.8).

Anal. Calcd. for $C_{14}H_{26}O_{16}Ba$: Ba, 23.37. Found: Ba, 23.08.

Cadmium D-Manno-D-*talo*-heptonate.—The cadmium salt was prepared from the pure lactone and cadmium carbonate. After three recrystallizations from 8 parts of hot water, the crystalline cadmium D-manno-D-*talo*-heptonate showed an $[\alpha]^{20}\text{D}$ value of +0.88° in water (*c* 1.2).

Anal. Calcd. for $C_{14}H_{26}O_{16}Cd$: Cd, 19.97. Found: Cd, 19.81.

D-Manno-D-*talo*-heptonic Amide.—D-Manno-D-*talo*-heptonic lactone (15 g.) was covered with liquid ammonia and stirred to complete solution. The solid (16 g., m.p. 173–174°) which resulted upon evaporation of the excess ammonia was recrystallized in 60% yield from 10 parts of 50% ethanol, and showed $[\alpha]^{20}\text{D}$ −11.6° in water (*c* 1.1), m.p. 175–176°.

Anal. Calcd. for $C_7H_{15}O_7N$: N, 6.22. Found: N, 6.20.

Octonic Series

Reaction of D-Manno-D-*gala*-heptose with Sodium Cyanide and Calcium Chloride.—Cooled solutions of 200 g. of D-manno-D-*gala*-heptose monohydrate in 1 l. of water, 55.8 g. of sodium cyanide in 200 ml. of water and 96.6 g. of calcium chloride dihydrate in 200 ml. of water were mixed and allowed to stand at 5° for 18 hours. Powdered calcium oxide (74 g.) was stirred into the solution and the mixture was heated on the steam-bath for two hours, then allowed to stand at 25° for 18 hours to complete the formation and precipitation of the basic calcium salts of the epimeric D-manno-L-*manno*- and D-manno-L-*gluco*-octonic acids. The basic calcium salts were collected by filtration and washed with limewater until the washings were chloride free. The washed basic salts were suspended in 1500 ml. of water, the mixture heated on the steam-cone, and carbon dioxide was passed in until the warm solution was neutral to phenolphthalein and then for one hour more. The mixture was filtered hot, the precipitate washed three times with 500 ml. of water, and the filtrate made up to four liters, cooled in ice, seeded with calcium D-manno-L-*manno*-octonate and allowed to stand at 5° for 24 hours. The precipitated salt was separated by filtration, washed twice with 200 ml. of water and dried at 50°. The dried salt was digested with five parts of water on the steam-bath for one hour to remove small amounts of calcium D-manno-L-*gluco*-octonate, then washed and dried. The average yield of pure **calcium D-manno-L-*manno*-octonate** was 55.5 g. (23%). The salt was recrystallized in a yield of 90% by solution in sixty parts of boiling water and addition of twenty parts of warm alcohol. It crystallized in micro-crystalline, thin, elongated platelets which showed a specific rotation $[\alpha]^{20}\text{D}$ of +9.2° in aqueous solution (*c* 0.56).

Anal. Calcd. for $C_{16}H_{30}O_{18}Ca$: Ca, 7.28. Found: Ca, 7.27.

Following the separation of the calcium D-manno-L-*manno*-octonate the filtrate was treated in either of two ways to recover derivatives of the epimeric D-manno-L-*gluco*-octonic acid. The calcium salt was obtained by concentrating the filtrate to 500 ml., warming it to 60–70° and adding 150 ml. of warm methanol. As the solution cooled, the **calcium D-manno-L-*gluco*-octonate** deposited as a seemingly gelatinous precipitate. The yield was about 160 g. (66%) and the salt was recrystallized by solution in 5 parts of warm water and the addition of 2 parts of methanol. In aqueous solution (*c* 1.00) the salt showed a specific rotation

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[13] H. S. Isbell, J. V. Karabinos, Harriet L. Frush, Nancy B. Holt, A. Schwebel and T. T. Galkowski, *J. Research Natl. Bur. Standards*, **48**, 163 (1952).

[14] All melting points were corrected.

[15] The $[\alpha]\text{D}$ value previously recorded (ref. 3) for barium D-manno-D-*gala*-heptonate, −9.5°, was not correct; the solvent was aqueous hydrochloric acid rather than water.

[16] G. Peirce, *J. Biol. Chem.*, **23**, 327 (1915).

[17] V. Ettel, *Collection Czechoslov. Chem. Communs.*, **4**, 504 (1932).

$[\alpha]^{20}D$ −3.5°. The salt was also prepared by addition of calcium hydroxide to a boiling aqueous solution of D-manno-L-*gluco*-octonic acid and showed a rotation of −3.3° in aqueous solution (*c* 0.66).

Anal. Calcd. for $C_{16}H_{30}O_{18}Ca$: Ca, 7.28. Found: Ca, 7.13.

The following alternate procedure gave D-manno-L-*gluco*-octonic acid directly. Following the separation by filtration of calcium D-manno-L-*manno*-octonate, the mother liquor was concentrated *in vacuo* (bath 50°) to a volume of about two liters, analyzed for calcium ions and sufficient 6 *N* sulfuric acid added to precipitate all the calcium. After 12 to 18 hours the precipitated calcium sulfate was removed by filtration and the mother liquor was concentrated *in vacuo* to a volume of 500 ml.; crystallization of the D-manno-L-*gluco*-octonic acid occurred as the concentration progressed. After cooling, the acid was collected by filtration and the mother liquor was concentrated further *in vacuo* to obtain a second crop. The crystalline **D-manno-L-*gluco*-octonic acid** weighed 105 g. (47%) and melted at 171–172°. It was recrystallized from 50% ethanol as clusters of rectangular plates, m.p. 171–172°. In aqueous solution (*c* 0.68) it showed mutarotation from an initial $[\alpha]^{20}D$ +7.0° to 0° in 2 hours and −14.0° in 18 hours and thereafter at a slower rate to a constant value of −17.2° in 7 days.

Anal. Calcd. for $C_8H_{16}O_9$: C, 37.50; H, 6.29; neut. equiv., 256. Found: C, 37.59; H, 6.45; neut. equiv., 260.

Pure **D-manno-L-*manno*-octonic phenylhydrazide** was prepared in a yield of 84% by heating D-manno-L-*manno*-octonic lactone with a slight excess of phenylhydrazine in very dilute acetic acid solution. It was recrystallized from 200 parts of boiling water in the form of irregularly shaped platelets which melted at 245–246°. A supersaturated solution of the compound in water at 50° (*c* 0.2) showed a rotation $[\alpha]^{50}D$ +17 ± 2° agreeing in sign with that expected from the phenylhydrazide rule. Fischer and Passmore[8] recorded a melting point of 243° under rapid heating, but did not measure the rotation.

Anal. Calcd. for $C_{14}H_{22}O_8N_2$: C, 48.55; H, 6.40; N, 8.09. Found: C, 48.43; H, 6.43; N, 7.89.

The epimeric **D-manno-L-*gluco*-octonic phenylhydrazide** was prepared by heating 3.0 g. of the acid, 3.6 ml. of phenylhydrazine, 2.1 ml. of acetic acid and 25 ml. of water on the steam-bath; yield 3.9 g. (95%), m.p. 219–220°. The compound was recrystallized from 50 parts of hot water and obtained as small prisms which rotated $[\alpha]^{50}D$ −12.8° in aqueous solution (*c* 0.41), m.p. 219–220°. Fischer and Passmore[8] did not describe this substance, but in the unpublished work of Hagenbach[18] there is mentioned a manno-octonic phenylhydrazide of m.p. 217–220° (224° cor.); its rotation was not recorded.

Anal. Calcd. for $C_{14}H_{22}O_8N_2$: C, 48.55; H, 6.40; N, 8.09. Found: C, 48.71; H, 6.36; N, 8.13.

D-Manno-L-*manno*-octonic Lactone from the Calcium Salt.—To a solution of 20 g. of finely ground calcium D-manno-L-*manno*-octonate in 500 ml. of water was added exactly 12 ml. of 6.094 *N* sulfuric acid. The mixture was refluxed for 15 minutes, cooled and filtered. The filtrate was concentrated to 100 ml., and any insoluble material was removed by filtration. Further concentration to dryness was effected by heating on a steam-bath in a current of air, and the residue was dissolved in 100 ml. of boiling 95% ethanol. After cooling, the lactone was collected by filtration and the crystals weighed 16.3 g. (94%), m.p. 172–174°, $[\alpha]^{20}D$ −43.5° (H_2O, *c* 2). Fischer and Passmore[8] recorded m.p. 167–170° and $[\alpha]^{20}D$ −43.6° for this lactone.

D-Manno-L-*manno*-octonic Lactone from the Phenylhydrazide.—To 10 g. of D-manno-L-*manno*-octonic phenylhydrazide previously warmed on the steam-bath under reflux with 20 ml. of ethanol and 20 ml. of water, was added 9 g. of copper sulfate pentahydrate in 80 ml. of water and the mixture refluxed for five hours. After removal of the copper as sulfide and the sulfuric acid by barium hydroxide the filtrate was concentrated to a sirup which crystallized on cooling; yield, crude lactone, 6.1 g. (88%). This was recrystallized from 95% ethanol, as in the previous experiment, to give material with similar constants.

Anal. Calcd. for $C_8H_{14}O_8$: C, 40.34; H, 5.92. Found: C, 40.47; H, 5.76.

Ethyl D-Manno-L-*manno*-octonate.—In a recrystallization of D-manno-L-*manno*-octonic lactone from hot ethanol a high-melting substance was obtained which, after further purification by several recrystallizations from water, gave m.p. 202–203° and $[\alpha]^{20}D$ −3.2° in water (*c* 0.5); the carbon and hydrogen analyses as well as a positive iodoform reaction indicate it to be the ethyl ester of D-manno-L-*manno*-octonic acid.

Anal. Calcd. for $C_{10}H_{20}O_9$: C, 42.25; H, 7.09. Found: C, 42.48; H, 7.17.

2-[D-*manno*-L-*gluco*-*octo*-Heptahydroxyheptyl]-benzimidazole.—A solution of 2.5 g. of D-manno-L-*gluco*-octonic acid, 1.15 g. of *o*-phenylenediamine (1.1 molecular equivalents) and 15 ml. of water was evaporated on the steam-bath to dryness; the dry, crystalline, orange-colored mass was then dissolved in 19.5 ml. of *N* hydrochloric acid (2 molecular equivalents), and the deep, red-colored solution was heated for 8 hours on the steam-bath, 10-ml. portions of water being added from time to time to replace that lost by volatilization, and finally the mixture was allowed to evaporate to dryness. The solid was dissolved in 5 ml. of cold water, the benzimidazole was precipitated by the addition of an excess of aqueous ammonia, filtered off and dried; yield 3.2 g. (quantitative) of a light pink powder.

The compound was recrystallized from 175 parts of water in the form of thin platelets which melted at 255–256° and rotated $[\alpha]^{20}D$ −6.0° in *N* hydrochloric acid (*c* 0.62), the sign of rotation being in agreement with that expected by the benzimidazole rule.[12]

Anal. Calcd. for $C_{14}H_{20}O_7N_2$: C, 51.21; H, 6.14; N, 8.53. Found: C, 51.29; H, 6.12; N, 8.31.

2[D-*manno*-L-*manno*-*octo*-Heptahydroxyheptyl]-benzimidazole.—This compound was prepared in essentially the same manner as its epimer except that D-manno-L-*manno*-octonic lactone was used. Following the 8-hour reaction period on the steam-bath, the product was dissolved in 15 ml. of water and the base precipitated by the addition of an excess of aqueous ammonia; yield 2.8 g. (82%). The benzimidazole was recrystallized from 250 parts of water and formed micro-crystalline needles which melted at 256–257° and rotated $[\alpha]^{20}D$ +13.6° in *N* hydrochloric acid solution (*c* 0.46), the sign of rotation again agreeing with that expected by the benzimidazole rule. It is relatively insoluble in the usual organic solvents. A mixed melting point with 2-[D-*manno*-L-*gluco*-*octo*-heptahydroxyheptyl]-benzimidazole, m.p. 255–256°, gave a value of 253–254°.

Anal. Calcd. for $C_{14}H_{20}O_7N_2$: C, 51.21; H, 6.14; N, 8.53. Found: C, 51.39; H, 6.05; N, 8.31.

Reaction of D-Manno-D-*gala*-heptose with Sodium Cyanide and Ammonium Chloride.—To 10 g. of D-manno-D-*gala*-heptose and 2.7 g. of ammonium chloride, dissolved in 30 ml. of distilled water, was added cracked ice to a volume of 70 ml. Sodium cyanide (2.3 g.) was added to the cold mixture and the whole maintained at 5°. Crystals of addition product began precipitating after 3 hours, and within 24 hours the precipitation was complete. The substance, identified as **ammonium D-manno-L-*manno*-octonate**, was collected by filtration, washed with ice-water and dried at 80°; yield 7.0 g. (54%), m.p. 165° with evolution of gas, $[\alpha]^{20}D$ +6° (water, *c* 1.6). Attempts to recrystallize the salt were unsuccessful.

Anal. Calcd. for $C_8H_{19}O_9N$: N, 5.1. Found: N, 5.2.

Conversion of Ammonium D-Manno-L-*manno*-octonate into D-Manno-L-*manno*-octonic Lactone.—The addition compound (6 g.) was dissolved in 25 ml. of distilled water and the solution was passed through a column of cation-exchange resin (Amberlite IR-100, 1.2 cm. in diameter and 40 cm. long) followed by 100 ml. of distilled water. The effluent solution was concentrated to dryness on the steam-bath. The sirupy residue began to crystallize upon seeding and the product was recrystallized from warm ethanol. D-Manno-L-*manno*-octonic lactone (4.2 g., 80%) was obtained with m.p. 168–170° and $[\alpha]^{20}D$ −40.1°. One recrystallization of the lactone from 25 parts of methyl cellosolve gave prisms, m.p. 172–174°, $[\alpha]^{20}D$ −43.5° in water (*c* 3), these

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[18] The original notebook of Dr. R. Hagenbach, who worked in Fischer's laboratory [see E. Fischer, "Untersuchungen über Kohlenhydrate und Fermente (1884–1908)," Verlag von Julius Springer, Berlin, 1909, p. 582, first footnote; also C. S. Hudson, *Advances in Carbohydrate Chem.*, **1**, 5 (1945)] was made available to us through the kindness of Professor H. O. L. Fischer.

constants agreeing with the previous values. These data indicate that the crystalline product was the ammonium salt of D-manno-L-*manno*-octonic acid.

Acetylation of Ammonium D-Manno-L-*manno*-octonate.—To 10 ml. of ice-cold acetic anhydride containing 0.5 ml. of concentrated sulfuric acid was added 1.5 g. of ammonium D-manno-L-*manno*-octonate. After complete solution, the mixture was allowed to stand at room temperature for 24 hours and then poured on 50 g. of cracked ice. Crystals were obtained which after several recrystallizations from ethanol appeared as flat plates with m.p. 148° and $[\alpha]^{20}D$ + 7° (chloroform, *c* 1.5); yield 0.2 g. The substance had the composition of a **D-manno-L-*manno*-octonic acid heptaacetate.**

Anal. Calcd. for $C_{22}H_{30}O_{16}$: C, 48.00; H, 5.49. Found: C, 48.03; H, 5.36; N, 0.0 (Dumas).

Sodium D-Manno-L-*gluco*-octonate.—To 13 g. of D-manno-L-*gluco*-octonic acid was added 25 ml. of 2 *N* sodium hydroxide. The salt was purified by recrystallization, in 80% yield, from 5 parts of water; $[\alpha]^{20}D$ −4.1° in water (*c* 1.2).

Anal. Calcd. for $C_8H_{15}O_9Na$: Na, 8.27. Found: Na, 8.19, 8.16.

D-Manno-L-*gluco*-octonic Amide.—Twenty grams of D-manno-L-*gluco*-octonic acid in 200 ml. of glacial acetic acid was refluxed for two hours (to a constant polarimeter reading of $[\alpha]^{20}D$ −55.6°). It was then transferred to a beaker, blown down to a thick sirup on the steam-bath and heated in an oven at 120° for 20 hours, yield 21 g. Five grams of this sirupy lactone was dissolved in liquid ammonia and the excess allowed to evaporate overnight at room temperature (hood). The crude amide was recrystallized from 25 ml. of water giving 2.7 g. of plates, m.p. 190–191°, $[\alpha]^{20}D$ −21.4° in water (*c* 1.6). The epimeric D-manno-L-*manno*-octonic amide, prepared by Hann, Maclay, Knauf and Hudson,[19] showed $[\alpha]^{20}D$ +9.8° in water, and the sign of rotation of each amide is thus in accord with the amide rule.

Anal. Calcd. for $C_8H_{17}O_8N$: C, 37.64; H, 6.72; N, 5.49. Found: C, 37.62; H, 6.68; N, 5.35.

D-Manno-L-*gluco*-octonic Amide Heptaacetate.—Five grams of amide was added to an ice-cold solution of 5 g. of anhydrous zinc chloride in 25 ml. of acetic anhydride. After standing in the ice-box overnight, the solution was poured on ice and extracted with chloroform. The extract was washed successively with aqueous sodium bicarbonate and water and after drying the solvent was evaporated. After a number of unsuccessful attempts to crystallize the sirupy amide heptaacetate from methanol and ethanol, it was finally obtained in crystalline form from benzene–petroleum ether; yield 3 g., m.p. 114–116°. Recrystallization of this material from ethanol gave crystals, m.p. 115–116°, optically inactive in chloroform (*c* 0.8).

Anal. Calcd. for $C_{22}H_{31}O_{15}N$: C, 48.09; H, 5.69; N, 2.55. Found: C, 47.82; H, 5.80; N, 2.55.

D-Manno-L-*gluco*-octonic Amide Octaacetate.—To 6 g. of amide was added an ice-cold solution of 3 ml. of concentrated sulfuric acid in 50 ml. of acetic anhydride. After standing one hour in the ice-box and two hours at room temperature, the amide went into solution and this was allowed to stand at 5° overnight. The solution was poured on ice and extracted in the usual manner with chloroform. Nine and one-half grams of crystals was obtained from methanol, m.p. 133–136°, which on recrystallization from ethanol gave m.p. 136–137°, $[\alpha]^{20}D$ −8.44° in chloroform (*c* 0.6).

Anal. Calcd. for $C_{24}H_{33}O_{16}N$: C, 48.73; H, 5.62; N, 2.37. Found: C, 48.57; H, 5.53; N, 2.39.

D-Manno-L-*manno*-octose.—A solution of 20 g. of D-manno-L-*manno*-octonic lactone in 200 ml. of water was reduced in the usual manner with 1000 g. of 2.5% sodium amalgam. The reduction mixture was filtered and neutralized with 10% sodium hydroxide and concentrated to 100 ml. This was poured into 350 ml. of 85% ethanol and allowed to cool. The salts were removed by filtration and the last trace of inorganic material was removed by allowing the filtrate to stand for one week. The filtrate was concentrated to 100 ml. and this solution was further purified by passing it through ion-exchange columns with 4 liters of water. Concentration of this solution to a sirup gave a semicrystalline mass after some time which could be purified by recrystallization from a mixture of 5 parts of water and 25 parts of glacial acetic acid. D-Manno-L-*manno*-octose was obtained as small prisms, m.p. 154°, mutarotating in water (*c* 2) from +11.7° to −7.5°; the mutarotation is recorded in Table I. A 1% solution of the octose was not fermented by bakers' yeast under conditions which favored the fermentation of D-glucose.

Anal. Calcd. for $C_8H_{16}O_8$: C, 39.99; H, 6.71. Found: C, 40.05; H, 6.68.

TABLE I

MUTAROTATION OF D-MANNO-β-L-*manno*-OCTOSE IN WATER (*c* 2, *l* 4) AT 20°

Time, min. after solution	$[\alpha]^{20}D$	$k_1 + k_2$ (min., decimal logs)
0	+11.7 extrap.	....
2	+11.3	....
5	+10.5	0.0063
11.5	+9.5	.0046
18.5	+8.1	.0049
30	+6.5	.0046
36	+5.5	.0047
43	+4.4	.0048
58	+2.5	.0049
75	+0.7	.0049
89	−0.6	.0050
114	−2.4	.0051
139	−3.9	.0052
205	−5.4	.0047
230	−6.2	.0051
292	−6.9	.0052
∞	−7.5	....
	Average	0.0049

D-Manno-L-*manno*-octonic Acid.—In one reduction of D-manno-L-*manno*-octonic lactone, where ion-exchange columns had not been employed, a substance crystallized which, after repeated recrystallization from 50% acetic acid, gave m.p. 173–174° and $[\alpha]^{20}D$ +5° mutarotating to −21° in 8 hours and −31° in 72 hours. This substance, which is apparently D-manno-L-*manno*-octonic acid, gave a depression in melting point to 165–167° on admixture with D-manno-L-*gluco*-octonic acid of m.p. 171–172°.

Anal. Calcd. for $C_8H_{16}O_9$: C, 37.50; H, 6.29; neut. equiv., 256. Found: C, 37.45; H, 6.25; neut. equiv., 250.

D-Manno-L-*manno*-octose Phenylhydrazone.—This compound was prepared according to the directions of Fischer and Passmore.[8] The phenylhydrazone, recrystallized from 200 parts of boiling water, gave m.p. 210–211°. Fischer and Passmore record a melting point of 212°.

Anal. Calcd. for $C_{14}H_{22}O_7N_2$: C, 50.90; H, 6.71. Found: C, 50.93; H, 6.81.

D-Manno-L-*manno*-octose Phenylosazone.—The osazone was prepared by heating a filtered solution of 2 g. of the phenylhydrazone in 500 ml. of water with a filtered solution of 12 ml. of phenylhydrazine, 12 ml. of acetic acid and 25 ml. of water for five hours. The yellow precipitate (0.3 g.) was collected by filtration, washed with water and dried; m.p. 227–228°, $[\alpha]^{20}D$ +32.3° (pyridine, *c* 0.4), $[\alpha]^{20}D$ +44.6° (methyl cellosolve, *c* 0.4). Fischer and Passmore[8] give m.p. 223°.

Anal. Calcd. for $C_{20}H_{26}O_6N_4$: C, 57.40; H, 6.26; N, 13.39. Found: C, 57.20; H, 6.26; N, 13.40.

D-Manno-L-*manno*-octose Phenylosotriazole.—D-Manno-L-*manno*-octose phenylosazone (4.8 g.) was refluxed with 4.2 g. of copper sulfate pentahydrate in 1500 ml. of water for five hours, according to the method of Hann and Hudson.[20] The hot filtered solution on cooling gave 1.2 g. of white crystals, m.p. 231–232°. Recrystallization from 300 parts of boiling water raised the m.p. to 244–245°, $[\alpha]^{20}D$ +77.3° in pyridine (*c* 0.3).

Anal. Calcd. for $C_{14}H_{19}O_6N_3$: C, 51.68; H, 5.89; N, 12.92. Found: C, 51.72; H, 5.94; N, 12.77.

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(19) R. M. Hann, W. D. Maclay, A. E. Knauf and C. S. Hudson, THIS JOURNAL, **61**, 1268 (1939).

(20) R. M. Hann and C. S. Hudson, *ibid.*, **66**, 735 (1944).

D-Manno-L-*manno*-octose Phenylosotriazole Hexaacetate.—One gram of the phenylosotriazole was dissolved in a mixture of 10 ml. of pyridine and 10 ml. of acetic anhydride. After standing for 24 hours, the solution was poured on ice. Recrystallization from ethanol of the precipitated solid yielded a total of 1.7 g. (95%), m.p. 101–102°, $[\alpha]^{20}_{\text{D}}$ +37.5° in chloroform (*c* 0.6).

Anal. Calcd. for $C_{26}H_{31}O_{12}N_3$: C, 54.07; H, 5.41; N, 7.28. Found: C, 54.37; H, 5.55; N, 7.15.

BETHESDA 14, MD.

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

A Convenient Synthesis of D-Manno-L-*manno*-octose

BY J. V. KARABINOS[1] AND C. S. HUDSON[2]

RECEIVED APRIL 8, 1953

The application of the Sowden–Fischer nitromethane synthesis for the preparation of D-manno-L-*manno*-octose is presented. The epimeric 1-nitro-1-desoxy-D-manno-L-*manno*-octitol and 1-nitro-1-desoxy-D-manno-L-*gluco*-octitol and their acetates have been prepared.

In the preceding paper[3] the synthesis of crystalline D-manno-L-*manno*-octose was reported, using the conventional sodium amalgam reduction of D-manno-L-*manno*-octonic lactone obtained from the hydrogen cyanide addition to D-manno-D-*gala*-heptose.

We now wish to report the successful application of the nitromethane addition method of Sowden and Fischer[4] to the synthesis of crystalline D-manno-L-*manno*-octose from the heptose with great improvement in yield and convenience. The addition of nitromethane to D-manno-D-*gala*-heptose was carried out with sodium methoxide in methanol solution, and from the reaction mixture the sodium salts of the epimeric C-nitroalcohols were precipitated. The free nitroalcohols were separated and characterized. 1-Nitro-1-desoxy-D-manno-L-*manno*-octitol was then converted to D-manno-L-*manno*-octose phenylhydrazone and crystalline D-manno-L-*manno*-octose was prepared from the hydrazone. The insoluble D-manno-L-*manno*-octose phenylhydrazone could also be isolated directly from the precipitated sodium salts of the epimeric C-nitroalcohols. In this manner the octose was prepared directly from the heptose in over-all yields of 15 to 19%.

Acknowledgment.—We are indebted to Mrs. Margaret M. Ledyard and Dr. W. C. Alford for microanalyses and to Mr. John Sipes for the preparation of a quantity of D-manno-D-*gala*-heptose. One of us (J.V.K.) held the Chemical Foundation Research Associateship while carrying out this research.

Experimental

Reaction of D-Manno-D-*gala*-heptose with Nitromethane.—To 50 g. of heptose suspended in 120 ml. of redistilled nitromethane and 190 ml. of methanol was added a solution of sodium methoxide prepared from 8.2 g. of sodium and 240 ml. of methanol and the mixture was mechanically shaken for 48 hours. The sodium salts of the epimeric C-nitroalcohols were collected by filtration at 5°, washed with cold methanol, and dried; weight 63 g. The tan, amorphous salts were dissolved in 500 ml. of water and passed through a column (6 × 80 cm.) of Amberlite IR-120. The eluate and washings were concentrated *in vacuo* to 600 ml. at which point crystallization occurred. After cooling overnight, the crystalline material was separated by filtration and amounted to 18 g. (28%), m.p. 186–191°.[5] Further concentration of the mother liquor to 100 ml. resulted in the separation of a second crystalline fraction, 6.5 g. (10%), m.p. 190–191°. The mother liquor and washings were combined and concentrated *in vacuo* to a sirup and then re-evaporated with absolute ethanol; finally 100 ml. of absolute ethanol was added and the sirup crystallized. Filtration followed by washing with ethanol and ether gave a third fraction, 27 g. (42%), m.p. 150–152°. Thus an 80% over-all yield of crude C-nitroalcohols was obtained.

After recrystallization of the first and second fractions twice from 60% ethanol and twice from water, a total of 12.6 g. (19.5%) of **1-nitro-1-desoxy-D-manno-L-*manno*-octitol** was obtained, $[\alpha]^{20}_{\text{D}}$ +5.8° in water (*c* 1.3), m.p. 191–192°.

Anal. Calcd. for $C_8H_{17}O_9N$: C, 35.43; H, 6.32; N, 5.16. Found: C, 35.42; H, 6.25; N, 4.97.

After four recrystallizations of the third fraction from 95% ethanol, 13.5 g. (21%) of the epimeric **1-nitro-1-desoxy-D-manno-L-*gluco*-octitol** was obtained, $[\alpha]^{20}_{\text{D}}$ +9.6° in water (*c* 1.9), m.p. 158–159°.

Anal. Calcd. for $C_8H_{17}O_9N$: C, 35.43; H, 6.32; N, 5.16. Found: C, 35.39; H, 6.30; N, 4.96.

1-Nitro-1-desoxy-D-manno-L-*manno*-octitol Heptaacetate.—One gram of 1-nitro-1-desoxy-D-manno-L-*manno*-octitol was acetylated in 8 ml. of acetic anhydride containing one drop of sulfuric acid by heating on the steam-bath for one-half hour. After pouring the mixture on ice, there was obtained 1.7 g. (93%) of crystalline heptaacetate. Recrystallization from 20 parts of ethanol gave needles, m.p. 135–136°, $[\alpha]^{20}_{\text{D}}$ −4.8° in abs. chloroform (*c* 2.4).

Anal. Calcd. for $C_{22}H_{31}O_{16}N$: C, 46.72; H, 5.53; N, 2.48. Found: C, 46.85; H, 5.39; N, 2.48.

1-Nitro-1-desoxy-D-manno-L-*gluco*-octitol Heptaacetate.—One gram of 1-nitro-1-desoxy-D-manno-L-*gluco*-octitol was acetylated in the same manner as the epimeric octitol. The sirupy product was obtained crystalline after standing in absolute ethanol at zero degrees and was recrystallized from ether–petroleum ether. The acetate gave m.p. 104–105° and showed $[\alpha]^{20}_{\text{D}}$ −3.8° in abs. chloroform (*c* 4.6).

Anal. Calcd. for $C_{19}H_{27}O_{14}N$: C, 46.72; H, 5.53; N, 2.48. Found: C, 46.59; H, 5.50; N, 2.42.

Conversion of 1-Nitro-1-desoxy-D-manno-L-*manno*-octitol into D-Manno-L-*manno*-octose Phenylhydrazone.—The higher-melting C-nitroalcohol (3.4 g.) was dissolved in 6.3 ml. of 2 *N* sodium hydroxide, and this solution added dropwise to a cold mixture of 3.2 ml. of sulfuric acid and 3.7 ml. of water. After neutralization with sodium carbonate monohydrate to congo red and with sodium bicarbonate to

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(1) Blockson Chemical Company, Joliet, Illinois.

(2) Deceased: Dec. 27, 1952.

(3) J. V. Karabinos, R. M. Hann and C. S. Hudson, THIS JOURNAL, **75**, 4320 (1953).

(4) J. C. Sowden and H. O. L. Fischer, THIS JOURNAL, **66**, 1312 (1944); **69**, 1963 (1947). Especially pertinent to the synthesis of higher-carbon sugars from D-mannose is the recently described preparation of D-manno-D-*gala*-heptose and D-manno-D-*talo*-heptose by this method; see J. C. Sowden and R. Schaffer, THIS JOURNAL, **73**, 4662 (1951).

(5) All melting points were corrected.