product was dissolved in a small amount of water and made alkaline with aqueous ammonia. The dark brown crystalline material that deposited was filtered and recrystallized several times from ethanol by the addition of water with sufficient activated carbon being used so that the final flaky crystalline product was practically colorless; the yield was 130 mg. Analyses showed the dibenzimidazole to be a monohydrate, thus confirming the earlier statement[12] that the enantiomorphous product derived from D-altroheptulosan (sedoheptulosan) was probably a monohydrate. Desiccation of the hydrate by heating for 5 hours at 100° *in vacuo* produced an anhydrous benzimidazole that melted at about 137° to a stiff sirup with subsequent characteristic liquefaction at about 160°. Identical values had been observed previously with specimens of the enantiomorphous dibenzimidazole derived from both D-altroheptulosan[12] and D-idoheptulosan.[6] Identical X-ray powder diffraction patterns (Table II and Fig. 1) also confirmed the enantiomorphous character of the dibenzimidazole hydrate derived from the D-series (D-altroheptulosan) and that from the new L-series (L-guloheptulosan). A comparison of their rotations will be discussed below.

Anal. Calcd. for $C_{18}H_{16}N_4O_3 \cdot H_2O$: N, 15.81; H_2O, 5.09. Found: N, 15.77; H_2O, 5.15. Calcd. for $C_{18}H_{16}N_4O_3$: C, 64.27; H, 4.80; N, 16.66. Found (dried 5 hours *in vacuo* at 100°): C, 64.49; H, 4.84; N, 16.59.

4-L-*glycero*-2-Hydroxymethyl-2,4-*cis*-di-(2-benzimidazolyl)-1,3-dioxolane Dihydrochloride Dihydrate and Its Enantiomorph.—When an attempt was made to measure the rotation in *N* hydrochloric acid of the dibenzimidazole derived from L-guloheptulosan, a crystalline hydrochloride separated almost immediately from the solution. The product, therefore, was filtered, and recrystallized from 80% ethanol by the addition of ether. The long needles melted at 237–245° (dec.). In *N* hydrochloric acid (*c* 1.1) the rotation $[\alpha]^{20}D$ +9.9° was equivalent to +13.1° when calculated as the anhydrous dibenzimidazole, and the latter is thus of the same magnitude but opposite in sign to the value −12.9° reported for the anhydrous dibenzimidazole derived from sedoheptulosan.[12]

For an additional comparison, about 50 mg. of the dibenzimidazole from the sedoheptulosan experiments[12] was converted similarly to its hydrochloride. The appearance of the product as long needles upon recrystallization from aqueous ethanol and ether, its m.p. of 239–247° (dec.), and its composition as a dihydrochloride dihydrate were in agreement with those expected for the enantiomorphous D-*glycero* form.

Anal. Calcd. for $C_{18}H_{16}N_4O_3 \cdot 2HCl \cdot 2H_2O$: C, 48.55; H, 4.98; N, 12.58; Cl, 15.92; H_2O, 8.09. Found (L-*glycero* form): C, 48.55; H, 5.05; N, 12.57; Cl, 16.07. (D-*glycero* form): C, 48.53; H, 5.16; N, 12.86; Cl, 15.95; H_2O (5 hours at 100° *in vacuo*), 8.59.

TABLE II

X-RAY POWDER DIFFRACTION PATTERN[a] OBTAINED FROM BOTH THE 4-D- AND THE 4-L-*glycero*-2-HYDROXYMETHYL-2,4-*cis*-DI-(2-BENZIMIDAZOLYL)-1,3-DIOXOLANE HYDRATES

Interplanar spacings, Å.	Relative intensities	Interplanar spacings, Å.	Relative intensities
17.1	5	4.38	2
8.45	4	4.24	2
7.7	3	4.02	2
7.0	1	3.83	4
6.15	2	3.66	3
5.75	3	3.49	2
5.45	4	3.36	3
5.1	3	3.22	1
4.80	2	2.83(B)	1
4.56	2		

[a] These data were obtained by the powder-wedge technique in a cylindrical camera with 7.16-cm. radius exposed to radiation from a copper anode X-ray tube with a nickel-foil filter giving essentially Cu Kα radiation. The relative intensities were estimated visually; 5 represents the strongest band, 1 the weakest band, and B a broad band.

Acknowledgment.—The authors wish to thank Mr. John T. Sipes for preparing the sedoheptulosan hydrate; Dr. James W. Pratt for assistance in hydrogenating it catalytically to volemitol and β-sedoheptitol; Mr. William C. White for the X-ray powder diffraction patterns; and Dr. William C. Alford, Miss Paula M. Parisius, Mrs. Evelyn G. Peake and Miss Mary Jean Stockton, all of this Institute, for carrying out the microchemical analyses.

BETHESDA 14, MARYLAND RECEIVED NOVEMBER 16, 1951

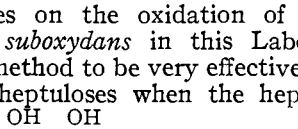
[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH, PUBLIC HEALTH SERVICE, FEDERAL SECURITY AGENCY]

D-Idoheptulose and 2,7-Anhydro-β-D-idoheptulopyranose[1]

BY JAMES W. PRATT, NELSON K. RICHTMYER AND C. S. HUDSON

The oxidation of D-gluco-D-*ido*-heptitol (synonym, D-ido-L-*gulo*-heptitol) to D-idoheptulose by *Acetobacter suboxydans* is in accord with the rule of specificity for that organism. The sirupy D-idoheptulose was characterized through its crystalline phenylosazone and phenylosotriazole, and its structure was proved through its catalytic hydrogenation to a mixture of D-gluco-D-*ido*-heptitol and the new ido-*ido*-heptitol. The action of acid on D-idoheptulose yielded a crystalline D-idoheptulosan whose structure was established, by periodate oxidation methods, as 2,7-anhydro-β-D-idoheptulopyranose.

Researches on the oxidation of heptitols by *Acetobacter suboxydans* in this Laboratory have shown the method to be very effective for the preparation of heptuloses when the heptitol has the

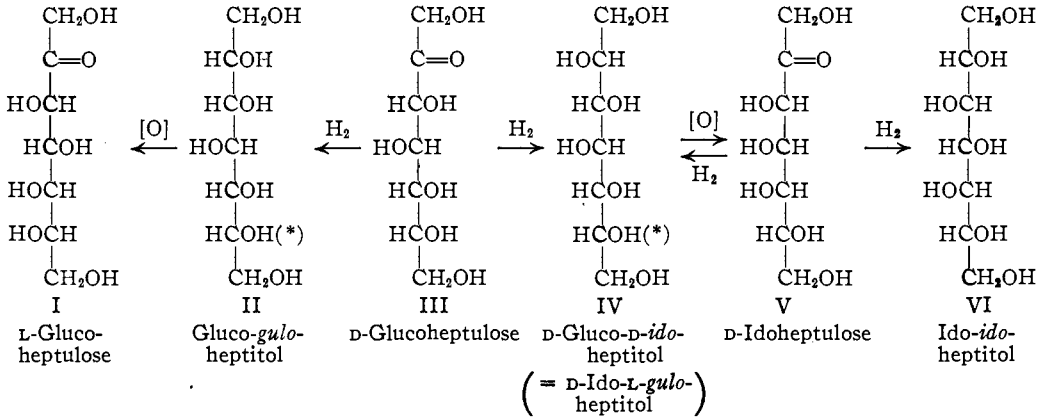


favorable configuration. Thus, perseitol (synonyms, D-manno-D-*gala*-heptitol and L-gala-D-*manno*-heptitol) was converted to crystalline L-galaheptulose (= perseulose)[2] and gluco-*gulo*-heptitol (II) (a *meso* form) was converted to crystalline L-glucoheptulose (I),[3] both sugars being produced in nearly quantitative yields. A third heptitol, volemitol (synonyms, D-manno-D-*talo*-heptitol and D-altro-D-*manno*-heptitol), has the favorable grouping at each end of its molecule and was found to be oxidizable by *A. suboxydans* to two ketoses, with crystalline D-mannoheptulose and sirupy D-altroheptulose (= sedoheptulose) being obtained

(1) A portion of this material has been taken from the thesis submitted by James W. Pratt to the Department of Chemistry of the Graduate School of Georgetown University in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1951.

(2) R. M. Hann, E. B. Tilden and C. S. Hudson, THIS JOURNAL, **60**, 1201 (1938); E. B. Tilden, *J. Bact.*, **37**, 629 (1939).

(3) W. D. Maclay, R. M. Hann and C. S. Hudson, THIS JOURNAL, **64**, 1606 (1942).



(*) Indicates point of attack by *Acetobacter suboxydans*.

in good yields and in about equal amounts.[4] Even more recently β-sedoheptitol (synonyms, D-altro-D-*gluco*-heptitol and L-gulo-D-*talo*-heptitol) has been oxidized to sirupy L-guloheptulose, whose structure has been proved in a definitive manner.[5]

We now wish to report our studies on the oxidation of a fifth heptitol, D-gluco-D-*ido*-heptitol (synonym, D-ido-L-*gulo*-heptitol) (IV). This substance was known earlier through reduction of the rare D-gluco-D-*ido*-heptose (= D-β-glucoheptose),[6] but we found a more convenient source in D-glucoheptulose (III); the catalytic hydrogenation of this ketose yielded a mixture of the heptitols II and IV from which the desired D-gluco-D-*ido*-heptitol (IV) could be separated by relatively simple procedures. The D-glucoheptulose (III), in turn, was prepared conveniently in about 50% yield by the rearrangement of D-gluco-D-*gulo*-heptose with lime-water. It is of interest to note that D-glucoheptose thus serves as starting material for both glucoheptuloses, for its hydrogenation yields directly gluco-*gulo*-heptitol (II) whose microbiological oxidation to L-glucoheptulose (I) has already been mentioned.

The action of *A. suboxydans* on D-gluco-D-*ido*-heptitol (IV) proceeded smoothly with the probably quantitative formation of a strongly reducing, levorotatory substance that proved to be D-idoheptulose (V). Although the sugar has so far remained a sirup, it was characterized through its crystalline phenylosazone and phenylosotriazole. Its formulation as D-idoheptulose was based, in part, upon its hydrogenation with Raney nickel as catalyst. One product was identified as the expected D-gluco-D-*ido*-heptitol (IV). The second product was a new crystalline heptitol that showed no optical activity even in aqueous ammonium molybdate,[7] and its crystalline heptaacetate was likewise inactive in chloroform. Of the three possible straight-chain *meso* heptitols, only gluco-*gulo*-heptitol (II) was known previously, and it was clearly different from our compound. To obtain the *meso* allo-*allo*-heptitol would have required that *A. suboxydans* oxidize two CHOH groups in the same polyhydric alcohol, a phenomenon that has never been reported. Only the predicted oxidation at C_6 in D-gluco-D-*ido*-heptitol (as written in formula IV) could account for the production of a third *meso* heptitol, and that could only be ido-*ido*-heptitol (VI), formed through hydrogenation of the intermediate D-idoheptulose (V). Further definitive proof that the sugar is D-idoheptulose will appear later in this paper.

Thus, the specificity rule of Bertrand[8] for the action of *Acetobacter xylinum* on polyhydric alcohols, as extended by Hann, Tilden and Hudson[2] to *A. suboxydans*, has again been confirmed by establishing D-idoheptulose (V) as the product of the action of *A. suboxydans* on D-gluco-D-*ido*-heptitol (IV). It seems probable, therefore, that the sirupy reducing substance that Cozic[9] obtained in the incomplete oxidation of D-gluco-D-*ido*-heptitol by *A. xylinum* also was D-idoheptulose; her product gave a positive Seliwanoff reaction but was not characterized by any crystalline derivative.

The second objective of our research was the study of the behavior of D-idoheptulose toward acids. It has long been known[10] that D-altroheptulose (= sedoheptulose) is converted under acidic conditions to an equilibrium mixture containing about 80% of a non-reducing anhydride, sedoheptulosan, whose structure has just recently been proved conclusively to be 2,7-anhydro-β-D-altroheptulopyranose (VIII).[11] The corresponding hexose, D-altrose, was later converted similarly to D-altrosan[12] to the extent of 57% and this anhydride was proved to be 1,6-anhydro-β-D-altropyranose (VII).[13] Aldoses with the D-idose configuration also were found to form anhydrides in the presence of acids, with D-idose being converted to 1,6-anhydro-β-D-idopyranose (IX)[14] in about 75%

(4) L. C. Stewart, N. K. Richtmyer and C. S. Hudson, *ibid.*, **71**, 3532 (1949); V. Ettel and J. Liebster, *Collection Czechoslov. Chem. Communs.*, **14**, 80 (1949).

(5) L. C. Stewart, N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **74**, 2206 (1952).

(6) L.-H. Philippe, *Compt. rend.*, **147**, 1481 (1908); *Ann. chim. phys.*, [8] **26**, 289 (1912).

(7) N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **73**, 2249 (1951).

(8) G. Bertrand, *Ann. chim. phys.*, [8] **3**, 202 (1904).

(9) M. Cozic, "Étude Biochimique de *Bacterium xylinum*," Imprimerie André Lesot, Nemours, 1933, p. 57; republished in *Rev. gén. botan.*, **46**, 157 (1934); see also Y. Khouvine, *Bull. soc. chim. biol.*, **18**, 1325 (1936).

(10) F. B. LaForge and C. S. Hudson, *J. Biol. Chem.*, **30**, 61 (1917).

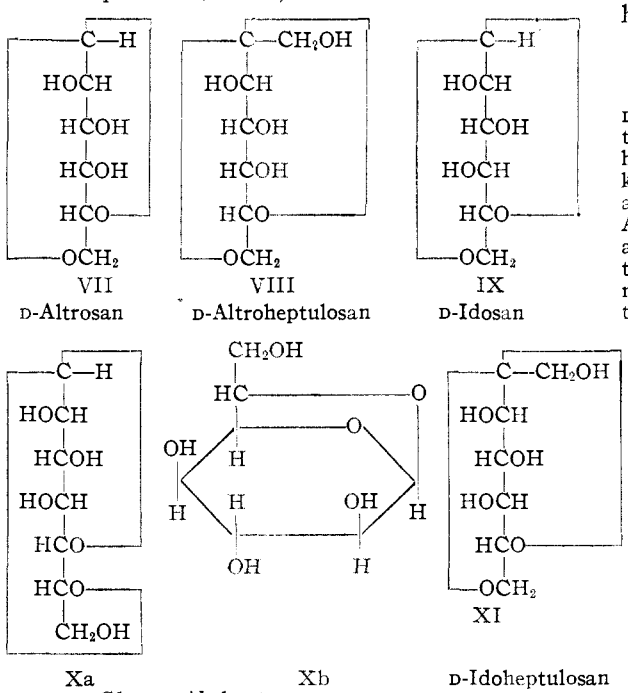
(11) J. W. Pratt, N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **74**, 2200 (1952).

(12) N. K. Richtmyer and C. S. Hudson, *ibid.*, **57**, 1716 (1935); **61**, 214 (1939).

(13) N. K. Richtmyer and C. S. Hudson, *ibid.*, **62**, 961 (1940).

(14) E. Sorkin and T. Reichstein, *Helv. Chim. Acta*, **28**, 1 (1945).

yield and D-gluco-D-*ido*-heptose being converted to 1,6-anhydro-D-gluco-β-D-*ido*-heptopyranose (Xa or Xb; the latter shows better the spatial arrangement of the anhydro ring)[15] in a 43% yield. It was of interest, therefore, to see whether D-idoheptulose would likewise form an anhydride. Our expectations were fulfilled, for one-hour heating with 0.2 *N* hydrochloric acid sufficed to decrease the reducing power of the D-idoheptulose solution to 15% of its original value, thus indicating its equilibrium with 85% of a non-reducing substance. The small amount of unchanged ketose was destroyed by heating with alkali, the solution was deionized, and the prismatic crystals obtained on subsequent concentration of the solution melted at 172–173°, showed $[\alpha]^{20}D$ −41.7° in water, and had the composition of an anhydroheptulose. The structure of this D-idoheptulosan was established through periodate oxidation methods. The consumption of two moles of oxidant per mole of compound, with accompanying liberation of one mole of formic acid, but no formaldehyde, indicated the presence of three contiguous secondary hydroxyl groups. The resulting dialdehyde showed the same rotation $[\alpha]^{20}D$ −16.9° that we had observed previously in the oxidation of sedoheptulosan (= D-altroheptulosan, VIII).[11] Further oxidation of



the dialdehyde with hypobromite yielded a dibasic acid that formed a crystalline calcium salt trihydrate whose rotations in water and in acid identified it with the salt obtained from sedoheptulosan.[11,16] Furthermore, condensation of the calcium salt with *o*-phenylenediamine and excess hydrochloric acid produced the same dibenzimidazole, as shown by rotation, melting point and mixed melting point, that we had obtained in our preceding study of sedoheptulosan.[11] Like tetratosylsedoheptulosan, the tetratosyl derivative of D-idoheptulosan did not react with sodium iodide when its acetone solution was heated for 65 hours at about 100° in a sealed ampoule. These data furnish definitive proof that D-idoheptulosan has the same ring system as D-altroheptulosan (VIII) and therefore must be written as 2,7-anhydro-β-D-idoheptulopyranose (XI). This proof serves also as confirmation that the action of *Acetobacter suboxydans* on D-gluco-D-*ido*-heptitol produced a 2-ketoheptose, namely, D-idoheptulose, and that the new *meso* heptitol mentioned earlier was indeed ido-*ido*-heptitol.

Inspection of formulas VII to XI, representing five sugar anhydrides, each with the same ring system and each having been formed by the action of acid on a sugar with a D-altrose or a D-idose configuration, reveals that the only feature they have in common, except for the rings, is the configuration of the first two of the three CHOH groups, counting from the top downward. However, even this observation no longer appears to have any special significance because L-guloheptulose, which has a *cis* rather than a *trans* arrangement for its corresponding groups, also forms a non-reducing anhydride readily under the influence of acids.[5]

Experimental

D-Glucoheptulose (III).—The rearrangement of D-gluco-D-*gulo*-heptose to D-glucoheptulose was improved by use of the following modification of Austin's procedure.[17] One hundred grams of the aldoheptose in 1 liter of limewater was kept at 35° for 7 days, the $[\alpha]^{20}D$ value changing to +37° in agreement with the two values +35° and +40° reported by Austin. To this mixture, at room temperature, were then added 100 g. of calcium carbonate and 14 ml. of bromine; the flask was shaken at intervals during the day. The next morning any excess bromine was expelled by aeration and the solution was filtered, deionized by passage through Amberlite IR-120 and Duolite A-4 ion-exchange columns and concentrated *in vacuo*. The rotation of the partially concentrated solution corresponded to a glucoheptulose content of 59 g., in good agreement with Austin's estimation of 60% ketose in the original equilibrium mixture. The solution was concentrated further to a moderately thick sirup that was taken up in 200 ml. of methanol and allowed to crystallize. The average yield, including material from combined mother liquors, was 50 g. of D-glucoheptulose of $[\alpha]^{20}D$ +65° to +67° in water. In case it is desirable to recover also the aldoheptonic acid, the aqueous solution, prior to its deionization, may be concentrated to 200 ml. and most of the calcium D-gluco-D-*gulo*-heptonate precipitated by the slow addition of 600 ml. of 95% ethanol.

Hydrogenation of D-Glucoheptulose (III) and Isolation of D-Gluco-D-*ido*-heptitol (IV).—This experiment was patterned after the method of Maclay, Hann and Hudson[3] for the reduction of the antipodal L-glucoheptulose. Thus, the solution obtained by shaking 200 g. of the D-enantiomorph in 1 liter of water with 40 g. of Raney nickel catalyst under hydrogen at 3000 p.s.i. (about 200 atmospheres) for 14 hours at 100° was filtered and concentrated *in vacuo* to a thick sirup that was dried by dissolving it in absolute ethanol and reconcentrating. The sirup was acetylated by adding 800 ml. of acetic anhydride and 50 g. of fused sodium acetate, and heating on the steam-bath; it was necessary to watch the temperature of the reaction mixture very carefully during the first hour (or until the sirup had dissolved completely) to keep the reaction from becoming too violent. The vessel was swirled gently from time to time to increase the rate of solution and plunged into an ice-bath whenever the temperature exceeded 110°. After two-hour additional

(15) J. W. Pratt, N. K. Richtmyer and C. S. Hudson, Abstracts of Papers, Chicago Meeting of the American Chemical Society, Sept. 8–8, 1950, page 10R.

(16) W. T. Haskins, R. M. Hann and C. S. Hudson, THIS JOURNAL, 74, 2198 (1952).

(17) W. C. Austin, *ibid.*, 52, 2106 (1930).

heating on the steam-bath, the mixture was poured over cracked ice and the portion of the gluco-*gulo*-heptitol heptaacetate that crystallized was removed by filtration. A second crop was obtained by neutralizing the filtrate with sodium bicarbonate, extracting with chloroform, concentrating the washed and dried extract to about 100 ml., adding 200 ml. of pentane, and allowing more gluco-*gulo*-heptitol heptaacetate to crystallize for several weeks in the refrigerator. The total over-all yield from D-glucoheptulose was 123 g. (26%).

The mother liquor was concentrated to a thick sirup that was dissolved in 600 ml. of methanol, cooled in a bath of ice and salt, and deacetylated by the addition of 10 ml. of a 3% solution of sodium methoxide. After two days in the refrigerator the crystalline material was separated by filtration and recrystallized twice from 0.5 part of hot water by the addition of 10 parts of hot methanol. In this way was obtained 43 g. of D-gluco-D-*ido*-heptitol, with an additional 19.5 g. (total, over-all yield 62.5 g., or 31%) being recovered from the mother liquors by reacetylation, removal of more gluco-*gulo*-heptitol heptaacetate, and subsequent deacetylation of the remainder of the product. The purified D-gluco-D-*ido*-heptitol, obtained as shiny, white, orthogonal platelets, melted at 128–129° and showed $[\alpha]^{20}D$ +1.20° in water (*c* 13.8), values that are comparable to the melting points of 130–131° and 129–130° and the rotations +0.8° and +0.7° reported by Philippe[6] and by Maclay, Hann and Hudson,[3] respectively. For its further characterization we have determined its rotation to be $[\alpha]^{20}D$ +114° in 5% ammonium molybdate solution (*c* 0.40) and $[\alpha]^{20}D$ +101° in the acidified molybdate solution (*c* 0.32) obtained by diluting 20 ml. of the preceding solution with 5 ml. of *N* sulfuric acid.[7]

Oxidation of D-Gluco-D-*ido*-heptitol (IV) by *Acetobacter suboxydans* to D-Idoheptulose (V).—A solution of 36 g. of D-gluco-D-*ido*-heptitol in 1800 ml. of water containing 0.5% of Difco yeast extract, 0.3% of potassium dihydrogen phosphate and 0.05% of D-glucose was distributed evenly among nine 2-liter erlenmeyer flasks, sterilized by autoclaving for 15 minutes at 120°, inoculated with 1 ml. per flask of a 72-hour culture of *Acetobacter suboxydans*,[18] and placed in an incubator at 30°. The reducing activity, which was estimated from time to time by the ferricyanide method of Hagedorn and Jensen as modified by Hanes,[19] reached a maximum in about 1 week, at which time the product showed a reducing value about 10% higher than that observed with D-mannoheptulose as a standard, and assuming complete conversion of the heptitol to heptulose. After 14-day incubation the solutions were combined and deproteinized by the method of Somogyi,[20] adding 250 ml. of a 20% aqueous solution of zinc sulfate heptahydrate followed by enough saturated aqueous barium hydroxide to bring the solution to about *p*H 7 (with brom thymol blue as indicator). About 20 g. of activated carbon was then added and the solution was filtered, deionized by passage through Amberlite IR-120 and Duolite A-4 ion-exchange resins, and concentrated *in vacuo* to 35 g. of a brownish sirup that did not crystallize. The rotation of this sirupy D-idoheptulose was estimated as $[\alpha]^{20}D$ −20° in water.

D-Idoheptose Phenylosazone.—A solution of 15.4 g. of D-idoheptulose sirup in a mixture of 140 ml. of water, 15 ml. of glacial acetic acid and 25 ml. of phenylhydrazine was heated on the steam-bath for 2 hours. Crystallization began within 15 minutes and appeared to be virtually complete in 30 minutes. The orange crystals, separated by filtration, washed with water, and dried in the air at 60°, weighed 21 g. The product was recrystallized readily from 8 parts of hot methyl cellosolve by the addition of 12 parts of boiling water. Upon cooling the solution, the phenylosazone separated as fine, yellow needles melting at 178–179° (dec.). Data on its mutarotation are recorded in Table I.

Anal. Calcd. for $C_{19}H_{24}N_4O_5$: C, 58.75; H, 6.23; N, 14.43. Found: C, 59.01; H, 6.43; N, 14.17.

D-Idoheptose Phenylosotriazole.—According to the method of Hann and Hudson,[21] 8.5 g. of D-idoheptose phenylosazone was refluxed for 1 hour in 850 ml. of water to which had been added 6 g. of cupric sulfate pentahydrate in 85 ml. of water. During the first 15 minutes the yellow osazone dissolved to give a deep red solution which gradually became green. Because no crystalline osotriazole appeared when the solution was concentrated in the usual way, the solution was next freed of copper and sulfate ions with hydrogen sulfide and barium carbonate, respectively. Concentration of the filtrate again failed to provide crystals, so the dark brown residue was taken up in 200 ml. of water and boiled for 5 minutes with 10 g. of activated carbon. The carbon cake was washed several times with water, then extracted thoroughly with hot acetone. Removal of the acetone in a current of air yielded 0.95 g. (15%) of crude D-idoheptose phenylosotriazole. On recrystallization from 20 parts of acetone the compound formed clusters of small needles melting at 120–122° and showing $[\alpha]^{20}D$ −44.9° in pyridine (*c* 0.84).

Anal. Calcd. for $C_{13}H_{17}N_3O_5$: C, 52.87; H, 5.80; N, 14.23. Found: C, 52.99; H, 5.75; N, 14.19.

TABLE I

MUTAROTATION OF D-IDOHEPTOSE PHENYLOSAZONE

In methyl cellosolve *c* 0.44, *l* 2		In pyridine *c* 1.4, *l* 2		In pyridine–ethanol (2:3) *c* 0.38, *l* 2	
Time	$[\alpha]^{20}D$	Time	$[\alpha]^{20}D$	Time	$[\alpha]^{20}D$
33 min.	±0.0°	5 min.	+11.6°	20 min.	−5.2°
		24 hr.	−35.5	24 hr.	−40.8
		48 hr.	−39.7		
72 hr. (constant)	−32.4	120 hr. (constant)	−43.4	96 hr. (constant)	−52.3

Hydrogenation of D-Idoheptulose (V) and Isolation of Ido-*ido*-heptitol Heptaacetate.—Sirupy D-idoheptulose obtained by the action of *Acetobacter suboxydans* on 9.8 g. of D-gluco-D-*ido*-heptitol was dissolved in 100 ml. of water and shaken with 3 g. of Raney nickel catalyst for 16 hours at 100° under hydrogen at 3000 p.s.i. The resulting solution, no longer reducing toward Fehling solution, was filtered and concentrated *in vacuo* to a thick sirup. The sirup was taken up in hot methanol, concentrated on the steam-bath in a stream of dry air, then dried by being stirred with hot benzene and concentrated again on the steam-bath. The resulting sirup was heated on the steam-bath with 2 g. of fused sodium acetate and 32 ml. of acetic anhydride for 3 hours; complete solution occurred during the first 30 minutes. The reaction mixture was poured on cracked ice; crystallization began within an hour and was allowed to continue overnight in the refrigerator. The crystalline material, recovered by filtration and washed with 15% aqueous acetic acid, weighed 11.5 g. (49%, based on the original D-gluco-D-*ido*-heptitol). It was recrystallized by dissolving in 12 parts of boiling methanol, adding activated carbon, filtering, and cooling. The very small prisms weighed 4.6 g., melted at 175–176°, and appeared to have a very slight positive rotation. A second recrystallization, from chloroform by the addition of pentane, did not change the melting point and the product showed no observable rotation in chloroform (*c* 2.4, *l* 4).

Anal. Calcd. for $C_{21}H_{30}O_{14}$: C, 49.80; H, 5.97; CH_3CO, 59.5. Found: C, 49.67; H, 6.07; CH_3CO, 59.6.

Ido-*ido*-heptitol (VI).—A sample of the heptaacetate weighing 3.3 g. was heated on the steam-bath with 250 ml. of methanol and 2 ml. of a 3% sodium methoxide solution until the acetate had dissolved completely. No crystals appeared when the solution was kept at 5° for 2 weeks, so the mixture was diluted with water, deionized, concentrated *in vacuo* to a small volume, and the product precipitated as a sirup by the addition of ethanol. From the sirupy phase crystals appeared after 2 days in the refrigerator. The 0.8 g. (58%) of heptitol thus obtained was recrystallized from a mixture of 5 ml. of methanol and 10 ml. of ethanol, separating in rosettes of chunky prisms that melted at 110–112° and showed no observable optical activity in 5% aqueous ammonium molybdate (*c* 0.4, *l* 4).

Anal. Calcd. for $C_7H_{16}O_7$: C, 39.62; H, 7.60. Found: C, 39.54; H, 7.30.

Isolation of D-Gluco-D-*ido*-heptitol (IV) from the Hydrogenated D-Idoheptulose.—The mother liquor remaining after the removal of the 11.5 g. of crude ido-*ido*-heptitol heptaacetate above was neutralized with sodium bicarbonate and extracted with chloroform. The washed and dried extract was concentrated, leaving a sirupy acetate that was dis-

(18) American Type Culture Collection No. 621.

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

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

(21) R. M. Hann and C. S. Hudson, THIS JOURNAL **66**, 735 (1944).

solved in methanol and deacetylated catalytically with sodium methoxide to yield 0.95 g. (10%, based on the original D-gluco-D-*ido*-heptitol) of D-gluco-D-*ido*-heptitol. After several recrystallizations from aqueous methanol the product was identified conclusively through its characteristic platelet form, its m.p. of 127–129° alone or mixed with the previously described original sample, and its $[\alpha]^{20}D$ value of +111° in 5% aqueous ammonium molybdate solution (*c* 0.33) as compared with the original $[\alpha]^{20}D$ +114° (*c* 0.40).[22]

D-Idoheptulosan (= 2,7-Anhydro-β-D-idoheptulopyranose) (XI).—In a preliminary experiment it was learned that D-idoheptulose is converted by hot 0.2 *N* hydrochloric acid within an hour to an equilibrium mixture that is more levorotatory than the original solution and has only about 15% of its original reducing power. Accordingly, 15.9 g. of a D-idoheptulose sirup in 200 ml. of 0.2 *N* hydrochloric acid was heated in a boiling water-bath for 1 hour. Fifteen grams of barium hydroxide octahydrate was added and the mixture heated in a beaker on the steam-bath to destroy the unchanged heptulose. Excess barium hydroxide was precipitated with carbon dioxide, and the remaining barium ions, chloride ions, and organic acids were removed by suitable ion-exchange columns. The aqueous solution was concentrated *in vacuo* to a thick sirup that was taken up in hot ethanol, treated with carbon, and cooled. The solution deposited 7.0 g. of non-reducing material that was recrystallized from 0.5 part of hot water by the addition of 10 parts of hot ethanol. The squat, somewhat irregular prisms of D-idoheptulosan melted at 172–173° and showed $[\alpha]^{20}D$ −41.7° in water (*c* 1.1).

Anal. Calcd. for $C_7H_{12}O_6$: C, 43.75; H, 6.30. Found: C, 43.97; H, 6.38.

Tetratosyl-D-idoheptulosan (= 1,3,4,5-Tetratosyl-2,7-anhydro-β-D-idoheptulopyranose).—To a solution of 1.5 g. of D-idoheptulosan in 100 ml. of dry pyridine was added 6.3 g. of *p*-toluenesulfonyl chloride and the mixture left at room temperature for 15 hours. The tosylated product was isolated with chloroform in the usual way, and the sirup obtained from the concentrated chloroform solution by precipitation with ethanol crystallized in part after the mixture had stood for 2 days in the refrigerator. The 1.3 g. (21%) of tetratosyl derivative was separated from the residual sirup, which probably contained considerable incompletely tosylated material, and was recrystallized as long needles from acetone by the cautious addition of water. The tetratosyl-D-idoheptulosan melted at 96–100° and showed $[\alpha]^{20}D$ −24.4° in chloroform (*c* 0.88).

Anal. Calcd. for $C_{35}H_{36}O_{14}S_4$: C, 51.97; H, 4.49; S, 15.85. Found: C, 52.06; H, 4.46; S, 16.02.

A 0.2343-g. sample of tetratosyl-D-idoheptulosan in 10 ml. of dry acetone containing 400 mg. of dry sodium iodide was sealed in a glass ampoule and heated in the cone of a steam-bath for 65 hours. No precipitate was obtained on cooling, and upon careful addition of water 0.2159 g. (92%) of the tetratosyl compound, identified by m.p. and mixed m.p., was recovered unchanged.

Oxidation of D-Idoheptulosan (XI) with Sodium Metaperiodate.—To a solution of 0.1423 g. of D-idoheptulosan in 10 ml. of water was added 5 ml. of an approximately 0.5 *M* solution of sodium metaperiodate and the volume adjusted exactly to 25 ml. with water. The optical rotation had become constant within 26 hours, and titrations at that time, as well as at the end of 4 days, showed that the consumption of oxidant was 2.02 moles per mole of glycosan with 0.98 mole of formic acid being liberated. No formaldehyde could be detected.

The final $[\alpha]^{20}D$ value of −16.9°, calculated as the expected dialdehyde, was observed in a similar experiment in which 4.99 g. of D-idoheptulosan was oxidized in 250 ml. of solution. The dialdehyde was freed from iodate and periodate by the addition of a slight excess of barium chloride, then oxidized for 15 hours at room temperature with 3 ml. of bromine in the presence of 12 g. of barium carbonate. Excess bromine was removed by aeration, halide ions with silver carbonate, silver ions with hydrogen sulfide, and barium ions by passage of the solution through a column of Amberlite IR-120 ion-exchange resin. The resulting solution was partially concentrated *in vacuo*, neutralized with aqueous calcium hydroxide to the phenolphthalein endpoint, and finally concentrated *in vacuo* to a thick sirup. Taken up in 10 ml. of hot water and brought to a point just below clouding with hot 95% ethanol, the material formed a thin paste of crystals when left overnight in the refrigerator. The filtered and air-dried product weighed 2.9 g. It was recrystallized from 30 parts of 50% ethanol in the form of fine needles weighing 1.7 g. It was identified as calcium 4-D-*glycero*-2-hydroxymethyl-1,3-dioxolane-2,4-*cis*-dicarboxylate trihydrate through its analysis and through its rotation of $[\alpha]^{20}D$ +42.4° in water (*c* 1.2) as compared with the previously reported values of +41.8°[11] and +43.5°[16] for the same product derived from sedoheptulosan. In *N* hydrochloric acid (*c* 0.55) it showed an $[\alpha]^{20}D$ value of +25.2°, calculated as the liberated dibasic acid, as compared with the +24° in *N* sulfuric acid (*c* 5.4) estimated previously[16] for the same product derived from sedoheptulosan.

Anal. Calcd. for $C_6H_6CaO_7 \cdot 3H_2O$: C, 25.35; H, 4.26; Ca, 14.10. Found: C, 25.57; H, 4.33; Ca, 14.31.

Additional identification was supplied by conversion of the calcium salt to the corresponding dibenzimidazole, namely, 4-D-*glycero*-2-hydroxymethyl-2,4-*cis*-di-(2-benzimidazolyl)-1,3-dioxolane, by the procedure described in a preceding paper.[11] The anhydrous product melted at about 137° to a stiff sirup that liquefied completely at about 160°; a mixture with the dibenzimidazole derived from sedoheptulosan showed no depression of these characteristic values. The anhydrous material showed $[\alpha]^{20}D$ −14.5 ± 2.4° in *N* hydrochloric acid (*c* 1) as compared with the −12.9 ± 1.5° reported previously.[11]

Anal. Calcd. for $C_{18}H_{16}N_4O_3$: C, 64.27; H, 4.80; N, 16.66. Found (dried 4 hours at 100° *in vacuo*): C, 64.35; H, 4.82; N, 16.43.

Acknowledgment.—The authors wish to thank Mr. John T. Sipes for preparing the D-gluco-D-*gulo*-heptose and rearranging it to the D-glucoheptulose used in these investigations; Dr. Laura C. Stewart for assistance in the *Acetobacter* oxidations; and Dr. William C. Alford, Miss Paula M. Parisius and Mrs. Evelyn G. Peake, all of this Institute, for carrying out the microchemical analyses.

BETHESDA 14, MD. RECEIVED DECEMBER 29, 1951

(22) Since this portion of the work was completed it was found (ref. 7) that rotations in this solvent vary with the concentration.