Anal. Calcd. for $C_{14}H_{21}O_{10}N$ (363.3): C, 46.3; H, 5.83. Found: C, 46.8; H, 5.88.

D-*arabo*-2-**Desoxyhexose** ("2-Desoxyglucose").[6]—One gram of the crude acetylated didesoxynitroalcohol, m. p. 84–86°, described above was dissolved at room temperature in 15 cc. of 1 *N* sodium hydroxide solution and allowed to stand for one hour. The solution was then added to a stirred mixture of 3 cc. of water and 2.1 cc. of sulfuric acid at room temperature. The solution was diluted and neutralized by stirring with barium carbonate. After centrifuging and filtering, the solution was treated with a few drops of acetic acid and was concentrated to dryness at reduced pressure. Treatment of the resulting sirup with 0.6 cc. of benzylphenylhydrazine in 10 cc. of 75% ethanol then yielded 0.68 g. (71%) of D-*arabo*-2-desoxyhexose benzylphenylhydrazone.[12] After recrystallization from ethyl acetate, the hydrazone melted at 158–159° and showed $[\alpha]^{26}D$ +7.6° in methanol, *c* 1.2.

Cleavage of the hydrazone with benzaldehyde according to the directions of Bergmann, Schotte and Lechinsky[7] gave the crystalline D-*arabo*-2-desoxyhexose, $[\alpha]^{23}D$ +46.6° (one hour) in water, *c* 2.

The desoxy sugar apparently was obtained as a mixture predominating in the α-form since its initial melting point of 128–129° was lowered to 123–125° on recrystallization and a slight downward mutarotation was evident in its aqueous solution: $[\alpha]^{23-25}D$ +53.8°, ten minutes; +50.3°, twenty minutes; +48°, thirty minutes; +46.6°, one hour (constant).

Acknowledgment.—The authors are pleased to acknowledge the support of the Corn Products Refining Company during the course of this research.

(12) Bergmann and Schotte, *Ber.*, **54**, 440 (1921).

Summary

Treatment of the acetylated carbohydrate C-nitroalcohols in benzene solution with sodium bicarbonate produces the corresponding acetylated nitroölefins in good yield.

Several unsubstituted sugars have been shown to condense with nitromethane in the presence of alkali. The products were isolated in crystalline form by conversion to the corresponding acetylated nitroölefins.

The acetylated nitroölefin arising from D-arabinose and nitromethane, D-*arabo*-tetraacetoxy-1-nitrohexene-1, was reduced catalytically to the acetylated 1,2-didesoxynitroalcohol. Deacetylation of this substance followed by treatment of the sodium nitroalcohol with sulfuric acid gave rise to D-*arabo*-2-desoxyhexose, ("2-desoxyglucose") in good yield. This new method of synthesis for 2-desoxy aldoses should prove valuable as a supplement to the previously known procedures for preparing this rare type of sugar.

TORONTO, CANADA RECEIVED DECEMBER 24, 1946

[CONTRIBUTION FROM THE CHEMISTRY LABORATORY, NATIONAL INSTITUTE OF HEALTH, U. S. PUBLIC HEALTH SERVICE]

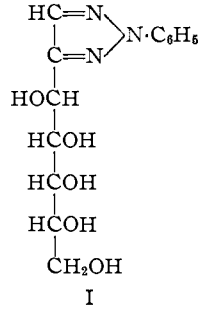
The Action of Copper Sulfate on the Phenylosazones of the Sugars. IV. The Phenylosotriazoles of Some Heptoses

BY W. T. HASKINS, RAYMOND M. HANN AND C. S. HUDSON

In previous articles[1] we have described the phenylosotriazoles of the pentoses and hexoses; the present article deals with the preparation of the phenylosotriazoles from four heptoses, namely, sedoheptulose (D-altroheptulose), D-mannoheptulose, D-gluco-D-*gulo*-heptose and D-gala-L-*gluco*-heptose and some of their acetyl and benzoyl derivatives. All of these heptose phenylosotriazoles crystallized readily and exhibited relatively low aqueous solubilities, especially D-*gala*-heptose phenylosotriazole, which has so low a solubility in water that it is comparable in this respect with D-*arabo*-hexose[2] phenylosotriazole. The new phenylosotriazoles, like the previous ones, are colorless very stable substances that possess sharp melting points and freedom from mutarotation, properties which make them valuable reference substances for establishing the identity of the respective phenylosazones.

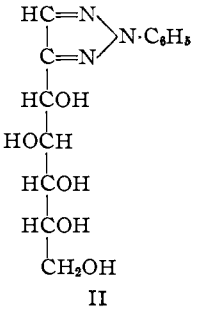
Oxidation of the heptose phenylosotriazoles by sodium metaperiodate produced in each instance a high yield (92–96%) of 2-phenyl-4-formyl-2,1,3-triazole (V) together with the appropriate amounts of formic acid and formaldehyde; these results show that the 4-substituted 2-phenyl-2,1,3-triazole structure that has been shown to be present in the pentose and hexose phenylosotriazoles also occurs in the heptose phenylosotriazoles.

The structures of the new phenylosotriazoles that have been proved by the oxidation of the substances with periodate are shown in the formulas.



I

Sedoheptulose phenylosotriazole
(D-*altro*-Heptose phenylosotriazole)

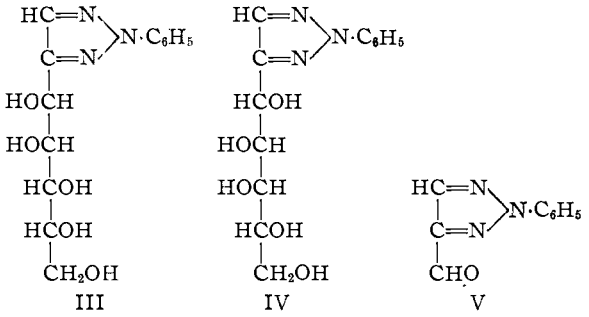


II

D-*gluco*-Heptose
phenylosotriazole

(1) Hann and Hudson, THIS JOURNAL, **66** 735 (1944); (II) Haskins, Hann and Hudson, *ibid.*, **67**, 939 (1945); (III) Haskins, Hann and Hudson, *ibid.*, **68**, 1766 (1946).

(2) There is here used the systematic nomenclature recently proposed by Sowden, *ibid.*, **69**, 1047 (1947).



III IV V

D-*manno*-Heptose phenylosotriazole | D-*gala*-Heptose phenylosotriazole | 2-Phenyl-4-formyl-2,1,3-triazole

We are indebted to Mr. Charles A. Kinser and Mrs. Betty Mount for carrying out the microchemical analyses.

Experimental

Sedoheptulose Phenylosotriazole (D-*altro*-**Heptose Phenylosotriazole**) (I).—To a suspension of 10 g. of finely ground sedoheptulose phenylosazone[3] (D-*altro*-heptose phenylosazone) in 900 ml. of boiling water was added a solution of 7.1 g. (1.1 molecular equivalents) of copper sulfate pentahydrate in 100 ml. of boiling water; the mixture was refluxed for one hour, filtered and concentrated *in vacuo* to about 100 ml., whereupon the product crystallized spontaneously. The concentrate was cooled to 5° for one hour, filtered and washed with cold water and cold alcohol to yield 4.7 g. (62%) of flattened lustrous needles. The compound was recrystallized from 25 parts of water or of alcohol and when pure it melted at 181–182° and rotated −71.5°[4] in pyridine (*c*, 0.81). It is soluble in warm methyl cellosolve, hot alcohol and hot water and nearly insoluble in chloroform, acetone and ethyl acetate.

Anal. Calcd. for $C_{13}H_{17}N_3O_5$: C, 52.87; H, 5.80. Found: C, 53.04; H, 5.99.

D-*altro*-**Heptose Phenylosotriazole Pentabenzoate.**—The benzoylation of D-*altro*-heptose phenylosotriazole in pyridine solution with benzoyl chloride in the usual way produced the pentabenzoate in 93% yield. It was recrystallized from a mixture of 10 parts of absolute alcohol and 5 parts of chloroform and it formed clusters of prismatic needles which melted at 114–115° and rotated +14.7° in chloroform (*c*, 0.88). It is soluble in hot alcohol, chloroform, acetone and pyridine and sparingly soluble in water and petroleum ether.

Anal. Calcd. for $C_{48}H_{37}N_3O_{10}$: C, 70.66; H, 4.57; C_6H_5CO, 64.4. Found: C, 70.55; H, 4.52; C_6H_5CO, 64.3.

D-*manno*-**Heptose Phenylosotriazole** (III).—The D-*manno*-heptose phenylosotriazole was prepared from D-*manno*-heptose phenylosazone[5] by the same method as previously described for the preparation of D-*altro*-heptose phenylosotriazole. The product crystallized spontaneously upon concentration of the reaction mixture to 100 ml. and was recovered by filtration in 80% yield; it was recrystallized from 20 parts of water or 60 parts of absolute alcohol and it formed long, fine needles which melted at 184–185° and rotated −27.5° in pyridine (*c*, 0.83). It is practically insoluble in chloroform, ether, acetone, cold alcohol and cold water and soluble in pyridine, warm methyl cellosolve and warm water.

(3) LaForge and Hudson, *J. Biol. Chem.*, **30**, 61 (1917).

(4) All of the crystalline compounds described were recrystallized to constant melting point and specific rotation; *c* is the concentration in grams per 100 ml. of solution; the tube length was 4 dm.; the melting points were observed with the stem of the thermometer immersed in the heated bath. The rotations refer in all cases to specific rotations, $[\alpha]^{20}D$.

(5) Fischer and Passmore, *Ber.*, **23**, 2226 (1890).

Anal. Calcd. for $C_{13}H_{17}N_3O_5$: C, 52.87; H, 5.80. Found: C, 52.71; H, 5.88.

D-*manno*-**Heptose Phenylosotriazole Pentaacetate.**—Acetylation of 1.0 g. of D-*manno*-heptose phenylosotriazole by warming it on the steam-bath for two hours with 8 ml. of acetic anhydride and 0.25 g. of fused sodium acetate and pouring the reaction mixture upon crushed ice gave 1.7 g. (quantitative) of its pentaacetate, which, upon recrystallization from 10 parts of chloroform by the addition of 60 parts of hexane, formed elongated prisms melting at 115–116° and rotating −5.0° in chloroform solution (*c*, 0.96). The pentaacetate is soluble in ether, acetone and warm methyl and ethyl alcohols and nearly insoluble in water and petroleum ether.

Anal. Calcd. for $C_{23}H_{27}N_3O_{10}$: C, 54.65; H, 5.38; CH_3CO, 42.6. Found: C, 54.71; H, 5.38; CH_3CO, 42.8.

D-*manno*-**Heptose Phenylosotriazole Pentabenzoate.**—The benzoylation of D-*manno*-heptose phenylosotriazole in the usual way gave an 82% yield of material which was recrystallized by dissolving in 2 parts of chloroform and adding 10 parts of absolute alcohol; this pentabenzoate crystallized slowly as clumps of fine needles which melted at 76–77° and rotated +41.1° in chloroform (*c*, 0.84). It is soluble in ether, acetone, pyridine and hot alcohol and only slightly soluble in water and hexane.

Anal. Calcd. for $C_{48}H_{37}N_3O_{10}$: C, 70.66; H, 4.57; C_6H_5CO, 64.4. Found: C, 70.86; H, 4.69; C_6H_5CO, 64.5.

D-*gluco*-**Heptose Phenylosotriazole** (II).—The treatment of D-*gluco*-heptose phenylosazone[6] in aqueous copper sulfate solution by the same procedure as that previously described for sedoheptulose phenylosazone, followed by concentration to 100 ml., gave a 79% yield of D-*gluco*-heptose phenylosotriazole, which was recrystallized from 20 parts of alcohol or 10 parts of water; it formed long, fine, silky needles which melted at 175–176° and rotated +46.9° in pyridine solution (*c*, 0.84). It is sparingly soluble in ether, chloroform, cold water and cold alcohol and soluble in pyridine, warm water and warm alcohol.

Anal. Calcd. for $C_{13}H_{17}N_3O_5$: C, 52.87; H, 5.80. Found: C, 53.06; H, 5.95.

D-*gluco*-**Heptose Phenylosotriazole Pentaacetate.**—The acetylation of D-*gluco*-heptose phenylosotriazole with fused sodium acetate and acetic anhydride by heating on the steam-bath for two hours gave the pentaacetate in quantitative yield. It was recrystallized by dissolving in 4 parts of chloroform and adding 40 parts of hexane; the clusters of stiff radiating needles melted at 111–112° and rotated +121.1° in chloroform (*c*, 0.83). It is soluble in ether, acetone, benzene and chloroform and insoluble in water and petroleum ether.

Anal. Calcd. for $C_{23}H_{27}N_3O_{10}$: C, 54.65; H, 5.38; CH_3CO, 42.6. Found: C, 54.78; H, 5.48; CH_3CO, 42.5.

D-*gluco*-**Heptose Phenylosotriazole Pentabenzoate.**—The pentabenzoate of D-*gluco*-heptose phenylosotriazole was obtained in quantitative yield; it was crystallized from its solution in 10 parts of alcohol and 1 part of chloroform as small compact prisms which melted at 110–112° and rotated +104.5° in chloroform (*c*, 0.93). It is soluble in chloroform, ether, acetone and pyridine and nearly insoluble in water and hexane.

Anal. Calcd. for $C_{48}H_{37}N_3O_{10}$: C, 70.66; H, 4.57; C_6H_5CO, 64.4. Found: C, 70.51; H, 4.60; C_6H_5CO, 64.1.

D-*gala*-**Heptose Phenylosotriazole** (IV).—The D-*gala*-heptose phenylosotriazole was prepared from D-*gala*-heptose phenylosazone[7] by the same method as that previously described for the preparation of D-*altro*-heptose phenylosotriazole; it is nearly insoluble in cold water and crystallizes at once when the reaction mixture is

(6) Fischer, *Ann.*, **270**, 77 (1892).

(7) Fischer, *ibid.*, **288**, 139 (1895).

cooled, as flocs of fine needles, in 70% yield. It was recrystallized from 100 parts of boiling water, from which it separated as fine silky needles which melted at 214–215° and rotated +80.3° in pyridine (*c*, 0.84). It is nearly insoluble in alcohol, chloroform, acetone, ether and cold water and moderately soluble in pyridine and hot water. It resembles D-*arabo*-hexose phenylosotriazole in ease of crystallization and low solubility in water, but the two substances can be distinguished readily by their melting points and rotations and of course their analyses are different.

Anal. Calcd. for $C_{13}H_{17}N_3O_5$: C, 52.87; H, 5.80. Found: C, 53.02; H, 5.83.

D-*gala*-**Heptose Phenylosotriazole Pentaacetate.**—The pentaacetate of D-*gala*-heptose phenylosotriazole was obtained in quantitative yield by warming 1 part of the phenylosotriazole with 0.25 parts of fused sodium acetate and 8 parts of acetic anhydride on the steam-bath for two hours and pouring the reaction mixture upon crushed ice. It crystallized from its solution in 5 parts of warm methyl alcohol as clusters of needles which melted at 134–135° and rotated +53.1° in chloroform solution (*c*, 0.92). It is soluble in warm methyl and ethyl alcohols, chloroform, acetone and ether and nearly insoluble in water and petroleum ether.

Anal. Calcd. for $C_{23}H_{27}N_3O_{10}$: C, 54.65; H, 5.38; CH_3CO, 42.6. Found: C, 54.78; H, 5.39; CH_3CO, 42.8.

D-*gala*-**Heptose Phenylosotriazole Pentabenzoate.**—The benzoylation of D-*gala*-heptose phenylosotriazole in pyridine solution with benzoyl chloride produced the pentabenzoate in quantitative yield. It was purified by crystallization from 20 parts of absolute alcohol and formed elongated prisms which melted at 134–135° and rotated +28.9° in chloroform solution (*c*, 0.81). It is soluble in ether, acetone, chloroform and pyridine and sparingly soluble in cold alcohol, water and hexane.

Anal. Calcd. for $C_{48}H_{37}N_3O_{10}$: C, 70.66; H, 4.57; C_6H_5CO, 64.4. Found: C, 70.85; H, 4.80; C_6H_5CO, 64.3.

Sodium Metaperiodate Oxidation of the Heptose Phenylosotriazoles.—The phenylosotriazoles from sedoheptulose, D-mannoheptulose, D-gluco-D-*gulo*-heptose and D-gala-L-*gluco*-heptose were oxidized by suspending a 0.4000 g. sample of each in 15 ml. of water and adding 4.5 molecular equivalents of 0.534 *M* sodium metaperiodate solution; the mixtures were shaken for three hours at 20°, cooled to 5° for two hours and the crystalline 2-phenyl-4-formyl-2,1,3-triazole recovered by filtration and washed with ice water; the yields in all cases were 92–96% of material melting at 68–69° and showing no depression of this value when admixed with an authentic sample of 2-phenyl-4-formyl-2,1,3-triazole. The aqueous solution and washings from the oxidation mixtures were diluted to 50 ml. and analyzed for formic acid, formaldehyde and consumed periodate; the results showed that the reaction for each heptose phenylosotriazole had followed the equation

$$C_{13}H_{17}N_3O_5 + 4NaIO_4 \longrightarrow C_9H_7N_3O + 3HCOOH + HCHO + 4NaIO_3 + H_2O$$

Accordingly, the structures of the four new phenylosotriazoles are those shown earlier in this article.

Summary

The D-*altro*-, D-*manno*-, D-*gluco*- and D-*gala*-heptose phenylosotriazoles and several of their acetyl and benzoyl derivatives are described. Their structures have been established through periodate oxidations.

BETHESDA, MARYLAND RECEIVED DECEMBER 4, 1946

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE OHIO STATE UNIVERSITY]

Degradative Studies on Streptomycin. I.[1]

BY I. R. HOOPER,[2] L. H. KLEMM,[2] W. J. POLGLASE[2] AND M. L. WOLFROM

Folkers and co-workers[3] have advanced the formula $(C_{21}H_{37-39}O_{12}N_7 \cdot 3HCl)_2 \cdot CaCl_2$ for the crystalline calcium chloride compound of streptomycin trihydrochloride. Later work[4] on mercaptolysis products favored the possibility with the higher hydrogen content. Our analytical data on purified samples of the calcium chloride compound of streptomycin trihydrochloride and on didesoxydihydrostreptobiosamine tetraacetate likewise substantiate the formula $C_{21}H_{39}O_{12}N_7$ for streptomycin. In addition we have established analytically the presence of one methyl group linked to carbon which must necessarily be a part of the portion $C_6H_8O_5<$ remaining after subtraction of the streptidine[5,6,7] and N-methyl-L-glucosamine[8] entities.

Hydrogenation of the calcium chloride compound of streptomycin trihydrochloride with Raney nickel catalyst at 150°, followed by methanolysis and acetylation yielded crystalline methyl dihydrostreptobiosaminide pentaacetate (m. p. 194–195° (cor.), $[\alpha]^{28}D$ − 123° in chloroform). Since methanolysis and acetylation of streptomycin[5] is known to yield a tetraacetate containing three methoxyl groups, it is evident that in the above compound a carbonyl group of streptomycin has been reduced to an alcohol group and this carbonyl group is the one that forms a dimethyl

(1) A preliminary notice of the work herein reported appeared in THIS JOURNAL, **68**, 2120 (1946).

(2) Bristol Laboratories Research Associate and Research Fellow (W. J. P.) of The Ohio State University Research Foundation (Project 224).

(3) R. L. Peck, N. G. Brink, F. A. Kuehl, Jr., E. H. Flynn, A. Walti and K. Folkers, THIS JOURNAL, **67**, 1866 (1945).

(4) F. A. Kuehl, Jr., E. H. Flynn, N. G. Brink and K. Folkers, *ibid.*, **68**, 2096 (1946).

(5) N. G. Brink, F. A. Kuehl, Jr., and K. Folkers, *Science*, **102**, 506 (1945).

(6) H. E. Carter, R. K. Clark, Jr., S. R. Dickman, Y. H. Loo, P. S. Skell and W. A. Strong, *ibid.*, **103**, 540 (1946).

(7) R. L. Peck, C. E. Hoffhine, Jr., Elizabeth W. Peel, R. P. Graber, F. W. Holly, R. Mozingo and K. Folkers, THIS JOURNAL, **68**, 776 (1946).

(8) F. A. Kuehl, Jr., E. H. Flynn, F. W. Holly, R. Mozingo and K. Folkers, *ibid.*, **68**, 536 (1946).