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°, $[\alpha]^{20}\mathrm{D}$ +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]

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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, $[\alpha]^{20}\mathrm{D}$ +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, $[\alpha]^{20}\mathrm{D}$ +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°, $[\alpha]^{20}\mathrm{D}$ −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 $[\alpha]^{20}\mathrm{D}$ −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.

litmus, the solution was acidified with one drop of acetic acid, and a solution of 1.4 ml. of phenylhydrazine in 3 ml. of 25% acetic acid was added. The D-manno-L-*manno*-octose phenylhydrazone which crystallized was collected by filtration, washed with water, ethanol and then ether. The yield was 3.5 g. (84.5%) of product melting at 207–209°.

Conversion of D-Manno-L-*manno*-octose Phenylhydrazone into D-Manno-L-*manno*-octose.—D-Manno-L-*manno*-octose phenylhydrazone (7.5 g.) was refluxed for 2.5 hours with 76 ml. of water, 7.6 ml. of benzaldehyde, 0.76 g. of benzoic acid and 15 ml. of ethanol. After cooling, the solution was decanted from benzaldehyde phenylhydrazone, the decantate extracted thrice with chloroform, and the aqueous layer concentrated *in vacuo* to a sirup which crystallized slowly after the addition of glacial acetic acid and seeding. The yield of octose, m.p. 150–152°, $[\alpha]^{20}\text{D}$ −7.4° (equilibrium) in water (c 3) amounted to 3.6 g. (66%).

Direct Preparation of D-Manno-L-*manno*-octose Phenylhydrazone and D-Manno-L-*manno*-octose Phenylosazone from D-Manno-D-*gala*-heptose and Nitromethane—Ten grams of heptose was shaken for 48 hours with nitromethane, sodium methoxide and methanol as described above. The sodium salts of the C-nitroalcohols were dissolved in 48 ml. of water, and added with stirring to a cooled mixture of 10 ml. of sulfuric acid and 12 ml. of water. After neutralization to congo red with sodium carbonate monohydrate and to litmus with sodium bicarbonate, the solution was acidified with acetic acid and a solution of 5 ml. of phenylhydrazine in 10 ml. of 25% acetic acid was added. The D-manno-L-*manno*-octose phenylhydrazone which crystallized was collected by filtration, washed with water, ethanol and ether; 3.6 g. (23%), m.p. 206–209°. In a subsequent larger run a 29% yield of phenylhydrazone was obtained.

To the mother liquor was added 8.5 ml. of phenylhydrazine and 6 ml. of glacial acetic acid and after heating on the steam-bath for three hours, the phenylosazone began to precipitate. After filtration and washing with ethanol and ether, the yield of D-manno-L-*manno*-octose phenylosazone amounted to 2.0 g. (10%).

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF WISCONSIN]

Isolation of Δ^7-Stigmastenol from Wheat[1]

BY D. R. IDLER,[2] A. A. KANDUTSCH[3] AND C. A. BAUMANN

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Δ^7-Stigmastenol has been isolated from the sterols of wheat germ oil by chromatography of the azoyl esters followed by crystallization. The sterol comprises 3% of the total sterols in this source. It appears to be one of the components of "α_3-sitosterol."

Δ^7-Sterols could be useful as starting materials for the introduction of the biologically important 11-keto group,[4,5] but the known examples are not abundant in sources that are readily available.[6–8] In the present study the azoyl esters of wheat sterols were separated into three zones by chromatography, and Δ^7-stigmastenol (Δ^7-spinastenol)[9] was isolated as the less soluble component of the middle zone. It comprised 3% of the original sterols. The upper zone sterols (8% of the total) appeared to be a mixture of other Δ^7-sterols. Thus wheat is at least as rich as oats[10] in this type of compound.

Derivatives of Δ^7-stigmastenol showed similarities in melting point and rotation to derivatives of α_3-sitosterol (Table I), an uncharacterized sterol isolated from wheat by crystallization,[11] and most of the infrared spectra of the two preparations were also similar. A significant difference, however, was a lack of absorption at 12.22 μ in the spectrum of Δ^7-stigmastenol, whereas α_3-sitosterol showed a moderately strong band in this region. Furthermore, the band at 11.25 μ was considerably weaker than the 11.46 μ band in the spectrum of Δ^7-stigmastenol, whereas the intensities were nearly equal in the spectrum of α_3-sitosterol (Fig. 1). In this connection the spectrum of the upper zone sterol is

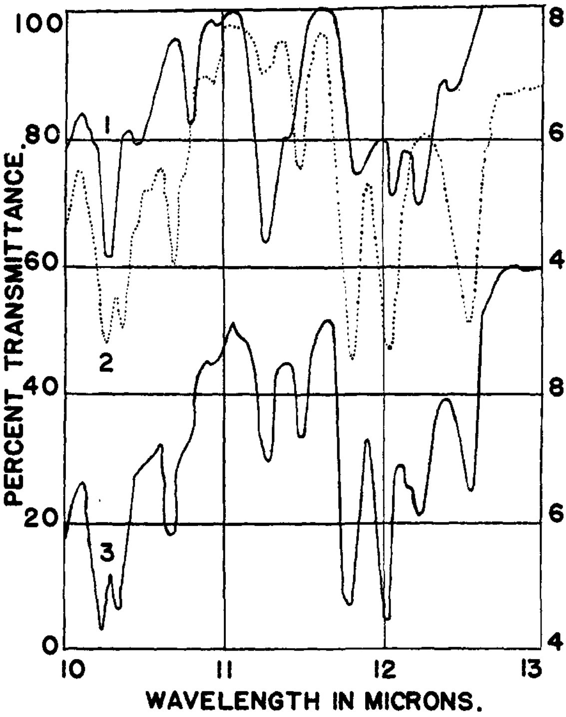

Fig. 1.—Comparison of the infrared spectra of the upper chromatographic zone of wheat sterols 1 (scale upper right), Δ^7-stigmastenol 2 (dotted line, scale on the left) and α_3-sitosterol 3 (scale lower right).

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(2) Babcock fellow, 1952–1953.

(3) Public Health research fellow of the National Cancer Institute, 1953–1954.

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(9) In the present discussion the product from wheat is referred to as 'Δ^7-stigmastenol" and the product prepared from α-spinasterol as "Δ^7-spinastenol."

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