

### Summary<sup>13</sup>

1. The resolution of racemic tartaric acid with 2-[D-glucosyl-D-gulo-hepto-hexahydroxyhexyl]-benzimidazole is described. The over-all yield of L-

(13) Postscript added March 31, 1939. We have been informed by Professor Karl P. Link that he and his students have been studying the preparation of benzimidazoles from sugar acids with particular reference to the use of these derivatives for the identification of sugars.

tartaric acid is over 90%.

2. A new method of preparation of benzimidazoles substituted in the [2] position with sugar residues is described. This method is based on the reaction of aldonic acids (or lactones) with *o*-phenylenediamine. The properties of a number of these substituted benzimidazoles are described.

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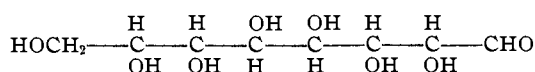
[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF HEALTH, U. S. PUBLIC HEALTH SERVICE]

## Relations between Rotatory Power and Structure in the Sugar Group. XXXI. The Configuration of D- $\alpha,\alpha$ -Mannooctose (D-Manno-L-manno-octose)<sup>1</sup>

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The establishment of the configuration of D- $\alpha,\alpha$ -mannooctose (D-manno-L-manno-octose)<sup>2</sup> is of considerable importance since this octose is the precursor of D- $\alpha,\alpha,\alpha$ -mannononose, which was reported by Fischer<sup>3</sup> in 1890 to be fermentable by yeast. In his published volume of collected works<sup>4</sup> he stated that a repetition of his earlier work by Hagenbach indicated the formation of other products when hydrogen cyanide acted upon the mannooctose, and mentioned his intention of repeating the research, but there is no further record.

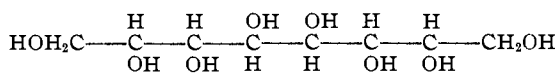
The accepted configuration of D- $\alpha,\alpha$ -mannooctose (I) is based upon Peirce's<sup>5</sup> recognition of



(I) D- $\alpha,\alpha$ -Mannooctose (D-Manno-L-manno-octose)

the configuration of the two D-mannoheptoses and his observation that the double lactone of D- $\alpha,\alpha$ -mannooctaric (D-manno-L-manno-octaric) acid upon dissolving in sodium hydroxide gave a solution which showed no detectable rotation. This proof is an application of Fischer's classical procedure of determining the configurations of the aldoses through comparison of the rotational relationships of their reduction products, the sugar alcohols, or the corresponding dibasic acids. Unfortunately the rotation in water of many of

the optically active alcohols is very small and recourse has been had to the substitution of saturated borax solution as a solvent to accentuate its value. Further, the low solubility of some of the higher alcohols prevents a reliable measurement; indeed Fischer prepared D- $\alpha,\alpha$ -mannooctitol, but did not report its rotation, undoubtedly because of its very low solubility. The question to be decided is, whether the alcohol possesses optical activity. If it does not, it must be a substance that is inactive by internal compensation, like xylitol and dulcitol, and must therefore possess the configuration (II).



(II) D- $\alpha,\alpha$ -Mannooctitol (D-Manno-L-manno-octitol)

Since this earlier work, methods have been developed which are of considerable aid in establishing configuration. The position of the hydrogen and hydroxyl groups about carbon two of the sugar chain may be assigned by utilization of the amide and the phenylhydrazide rules. It has also been shown that conversion of the sugar alcohols to their acetates offers a particular advantage in solving questions of configuration since the alcohol acetates in general are highly soluble in certain organic solvents and have rotations of considerable magnitude; thus Hockett and Hudson<sup>6</sup> found that D-arabitol, which requires borax to augment its rotation to a measurable value, upon acetylation yields a pentaacetate rotating +37.2° in chloroform.

It seemed desirable to supplement Peirce's proof

(1) Publication authorized by the Surgeon General, U. S. Public Health Service. No. XXX was published in THIS JOURNAL, 52, 2534 (1930).

(2) Concerning this nomenclature see Hudson, *ibid.*, 60, 1537 (1938).

(3) Fischer, *Ber.*, 23, 2238 (1890).

(4) Fischer, "Untersuchungen über Kohlenhydrate und Fermente," 1, 582 (1909).

(5) Peirce, *J. Biol. Chem.*, 23, 327 (1915).

(6) Hockett and Hudson, THIS JOURNAL, 57, 1753 (1935).

of the configuration of D- $\alpha$ , $\alpha$ -manno-octose by application of a method which would allow definitive allocation of the hydroxyl group on carbon two of the molecule. Upon treatment of D- $\alpha$ , $\alpha$ -manno-octonic lactone with liquid ammonia by the method of Glattfeld and MacMillan,<sup>7</sup> a crystalline manno-octonic amide rotating  $+9.6^{\circ}$ <sup>8</sup> was obtained, thus showing that the hydroxyl group is to the right. The reduction of sirupy D- $\alpha$ , $\alpha$ -manno-octose with hydrogen in the presence of Raney nickel produces crystalline D- $\alpha$ , $\alpha$ -manno-octitol and since this substance is too insoluble for rotatory measurement it was converted into its crystalline octaacetate, which, as expected, proved to be optically inactive.

The results by the two independent methods therefore confirm Peirce's assignment of the structure of D-manno-L-manno-octose to Fischer's D- $\alpha$ , $\alpha$ -manno-octose. It follows, then, that the configuration of Fischer's D- $\alpha$ , $\alpha$ -manno-octose must be that of either D-manno-L-gala-nonose or D-manno-L-talo-nonose. It seems remarkable that a sugar of either of these configurations is fermentable by yeast.

We express our appreciation to Dr. W. T. Haskins, who performed the micro analyses recorded in this study.

### Experimental

**D- $\alpha$ , $\alpha$ -Manno-octonic Amide (D-Manno-L-manno-octonic Amide).**—A solution of 5 g. of D- $\alpha$ , $\alpha$ -manno-octonic lactone in 15 cc. of liquid ammonia was allowed to evaporate spontaneously at room temperature; it yielded a friable crystalline mass of amide; yield 5.3 g. (quantitative). The substance was recrystallized by solution in 20 parts of hot water, separating in colorless micro-crystalline needles rotating  $+9.8^{\circ}$  in water (*c*, 0.53). The melting point upon rapid heating was 218–219°.

*Anal.* Calcd. for C<sub>8</sub>H<sub>12</sub>O<sub>3</sub>N: C, 37.62; H, 6.72; N, 5.49. Found: C, 37.51; H, 6.72; N, 5.37.

**D- $\alpha$ , $\alpha$ -Manno-octonic Amide Octaacetate (D-Manno-L-manno-octonic Amide Octaacetate).**—Acetylation of the amide was carried out by the procedures of Robbins and Upson.<sup>9</sup> Six grams of finely powdered D- $\alpha$ , $\alpha$ -manno-octonic amide was added to an ice-cold mixture of 50 cc. of acetic anhydride and 3 cc. of concentrated sulfuric acid. After standing for one hour in a freezing mixture, the suspension was allowed to stand at room temperature to complete solution. After standing overnight the solution was poured upon crushed ice and the crystalline precipitate separated by filtration; yield 10.2 g. (73%). The amide octaacetate was recrystallized from 10 parts of ethyl al-

cohol, separating in shining plates melting at 172–173° (corr.) and rotating  $+15^{\circ}$  in chloroform (*c*, 2.19).

*Anal.* Calcd. for C<sub>24</sub>H<sub>38</sub>O<sub>10</sub>N: C, 48.71; H, 5.62; N, 2.37. Found: C, 48.67; H, 5.94; N, 2.38.

**D- $\alpha$ , $\alpha$ -Manno-octonic Amide Heptaacetate (D-Manno-L-manno-octonic Amide Heptaacetate).**—Five grams of finely powdered amide was added to an ice-cold solution of 25 cc. of acetic anhydride containing 5 g. of fused zinc chloride. After one hour the suspension was removed from the freezing mixture and allowed to stand at room temperature; it gradually warmed and the amide dissolved. After standing overnight the solution was poured upon crushed ice and a fraction of 2.6 g. of the amide octaacetate melting at 170–171° and rotating  $+14.9^{\circ}$  in chloroform was removed. The mother liquors were extracted with chloroform in the usual way and upon concentration yielded 2.7 g. of a dry sirup. By three successive recrystallizations from 5 parts of alcohol a yield of 0.7 g. of D- $\alpha$ , $\alpha$ -manno-octonic amide heptaacetate of melting point 99–100° (corr.) and rotation of  $-15.9^{\circ}$  in chloroform (*c*, 1.00) was obtained.

*Anal.* Calcd. for C<sub>24</sub>H<sub>38</sub>O<sub>10</sub>N: C, 48.06; H, 5.69; N, 2.55. Found: C, 47.93; H, 5.86; N, 2.55.

**D- $\alpha$ , $\alpha$ -Manno-octitol (D-Manno-L-manno-octitol).**—A sirup prepared by the sodium amalgam reduction of 20 g. of D- $\alpha$ , $\alpha$ -manno-octonic lactone was further reduced by hydrogen at a pressure of 133 atmospheres and a temperature of 98° in the presence of Raney nickel. The resulting crystalline alcohol was recrystallized from 100 parts of boiling water, forming colorless plates which melted at 262–263° (corr.). It was not possible to measure the rotation in water because of its very low solubility.

*Anal.* Calcd. for C<sub>8</sub>H<sub>18</sub>O<sub>3</sub>: C, 39.65; H, 7.49. Found: C, 39.81; H, 7.35.

**D- $\alpha$ , $\alpha$ -Manno-octitol Octaacetate (D-Manno-L-manno-octitol Octaacetate).**—One gram of the alcohol was refluxed with a mixture of 35 cc. of acetic anhydride, 0.5 g. of fused sodium acetate and a trace of fused zinc chloride for six hours, solution gradually occurring during this period. The acetate was precipitated in quantitative yield by pouring into ice water and was then recrystallized from 25 parts of 95% alcohol, being obtained in colorless needles melting at 166–167° (corr.), which were optically inactive in chloroform solution (*c*, 1.2).

*Anal.* Calcd. for C<sub>24</sub>H<sub>34</sub>O<sub>10</sub>: C, 49.80; H, 5.93; CH<sub>3</sub>CO, 59.52. Found: C, 49.82; H, 5.95; CH<sub>3</sub>CO, 59.76.

### Summary

Confirmation of Peirce's allocation of the configuration of D-manno-L-manno-octose to Fischer's D- $\alpha$ , $\alpha$ -manno-octose has been obtained by two independent methods, namely, (1) proof that the hydroxyl group on carbon two is to the right (since D- $\alpha$ , $\alpha$ -manno-octonic lactone upon treatment with liquid ammonia yields a positively rotating amide), and (2) demonstration of the optical inactivity of D- $\alpha$ , $\alpha$ -manno-octitol octaacetate.

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(7) Glattfeld and MacMillan, *THIS JOURNAL*, **56**, 2481 (1934).

(8) All rotations are constant specific rotations at 20° for sodium light, *c* is concentration in grams in 100 cc. of solution; the tube length was 4 dcm.

(9) Robbins and Upson, *THIS JOURNAL*, **60**, 1788 (1938).