# BROMINE OXIDATION AND MUTAROTATION MEASUREMENTS WITH α-d-β-MANNOHEPTOSE AND α-d-α-GULOHEPTOSE

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### ABSTRACT

The lead salts of d-α-mannoheptonic acid and d-β-mannoheptonic acid were made and used for the separation of the corresponding free acids. The gamma lactone of d-β-mannoheptonic acid was prepared and reduced with sodium amalgam to give d-β-mannoheptose which was separated in the crystalline alpha modification. This new sugar is structurally related to α-d-talose and exhibits similar properties. Its mutarotation is complex, consisting in a fast change followed or accompanied by a smaller slow change. The proportions of the constitutents involved in the rapid reaction vary with temperature so that a change in temperature results in a rapid mutarotation. The temperature coefficient for the rapid mutarotation reaction corresponds to that for the rapid mutarotation reactions of galactose, arabinose, talose, ribose, and d-β-glucoheptose, whereas the temperature coefficient for the slow change agrees with the temperature coefficients for the mutarotations of glucose, mannose, gulose, and other reactions which consist in the interconversion of the alpha and beta pyranoses. The parallelism between the properties of d-talose, d-α-guloheptose, and d-β-mannoheptose is evidence that the configurations of the five carbon atoms comprising the pyranose ring determine in large measure the composition of the equilibrium solutions of the sugar. Measurements of the rates of oxidation with bromine water show that α-d-β-mannoheptose and α-d-α-guloheptose are oxidized at rates comparable to that previously found for the oxidation of α-d-talose and that the equilibrium solutions contain substantial quantities of rapidly oxidizable material.

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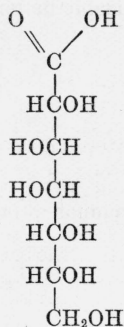## I. MUTAROTATION OF α-d-β-MANNOHEPTOSE

The mutarotations of many sugars reveal that when the sugar is dissolved in water, at least two fundamentally different reactions occur [1, 2, 3].[1] One of these consists in the interconversion of the

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[1] The numbers in brackets throughout this paper relate to the literature citations on page 108.

alpha and beta pyranose modifications, while the other involves labile modifications of unknown structure. The interconv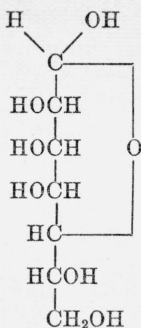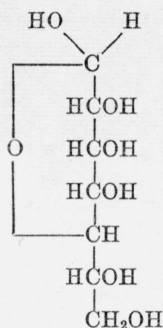ersion of the alpha and beta pyranoses occurs relatively slowly and the equilibrium state is not influenced markedly by temperature changes. The other reaction involving the labile modification is more rapid and is characterized by variation of the equilibrium position with temperature [4]. Mutarotation and bromine oxidation measurements [3] with sugars containing the glucose, mannose, and gulose structures show that the equilibrium solutions of these sugars consist almost exclusively of the alpha and beta pyranose modifications. On the other hand, the mutarotation and oxidation measurements [3, 5] with sugars containing the galactose, talose, and idose structures show that the equilibrium solutions of these sugars contain substantial proportions of modifications other than the alpha and beta pyranoses. The changes in optical rotation caused by the formation of the labile constituents in the solutions of galactose, arabinose, and related sugars are small and therefore it is difficult to study these interesting reactions by observations on these readily available sugars. The changes in optical rotation caused by the formation of the labile constituents in solutions of talose are much larger and therefore this sugar is more suitable for use in investigating this subject. However, talose is difficult to prepare [6] and therefore its application is restricted. In a previous paper [7], the author reported the preparation of $d$-$\alpha$-guloheptose, which has the talose structure and is also suitable for investigating these labile reactions. This sugar, however, is prepared from the synthetic sugar, $d$-gulose, so that its preparation represents a long and tedious process. A second heptose, $d$-$\beta$-mannoheptose, containing the talose structure, can be made from mannose. The preparation of this sugar was undertaken with the hope that it would provide a more readily available sugar for the investigation of the character of the labile modification of the sugars and the rapid mutarotation reactions.

<pre>
        O    OH                    O    OH
         \\  /                      \\  /
           C                          C
           |                          |
         HCOH                       HOCH
           |                          |
         HOCH                       HOCH
           |                          |
         HOCH                       HOCH
           |                          |
         HCOH                       HCOH
           |                          |
         HCOH                       HCOH
           |                          |
        CH₂OH                      CH₂OH
</pre>

  I. $d$-$\alpha$-Mannoheptonic acid          II. $d$-$\beta$-Mannoheptonic acid

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      H     OH                         HO     H
       \   /                             \   /
         C                                 C
         |                                 |
       HOCH                              HCOH
         |                                 |
       HOCH    O                     O   HCOH
         |                                 |
       HOCH                              HCOH
         |                                 |
       HC                                 CH
         |                                 |
       HCOH                              HCOH
         |                                 |
       CH₂OH                            CH₂OH
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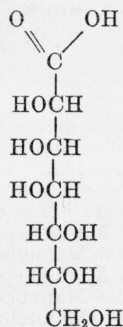
III.  α-d-β-Mannoheptose        IV.  α-d-α-Guloheptose

Application of the cyanhydrin synthesis to mannose [8] gives a large quantity of $d$-α-mannoheptonic acid, I, and a small quantity of $d$-β-mannoheptonic acid, II. But since $d$-α-mannoheptonic acid can be converted to $d$-β-mannoheptonic acid, by rearrangement in pyridine solution, considerable quantities of $d$-β-mannoheptonic acid can be prepared without much difficulty. The most tedious part of the process is the separation of the two acids. Several salts of these acids have been studied as a possible means for accomplishing this separation. The purification of $d$-α-mannoheptonic acid is not laborious because it forms difficultly soluble barium [9] and calcium [10] salts. On the other hand, $d$-β-mannoheptonic acid forms a difficultly soluble lead salt from which the free acid can be prepared. The free acid forms a crystalline γ-lactone, which, on reduction with sodium amalgam, gives α-$d$-β-mannoheptose, III. This sugar separates in long, thin, wedge-shaped crystals which melt at 83° C and give $[\alpha]_D^{20}=+45.7$. The analysis reveals that the new sugar is a monohydrate, and since the oxygen ring lies to the right [11] and it is the more dextrorotatory member of the alpha-beta pair, it is designated α-$d$-β-mannoheptose monohydrate.

When dissolved in water, α-$d$-β-mannoheptose exhibits the complex mutarotation given in table 1. This mutarotation can be represented by the following equations:

$$[\alpha]_D^{20}=+14.5+8.3\times10^{-.0141t}+22.9\times10^{-.0916t}$$

$$[\alpha]_D^{0.1}=+16.5+9.3\times10^{-.00181t}+19.3\times10^{-.0159t}$$

in which $t$ is the time measured in minutes after the sugar is dissolved in water. The complex mutarotation shows that the equilibrium is established between certain modifications more rapidly than between the others, and that the solution contains at least three modifications of the sugar in dynamic equilibrium. Inspection of the data and equations representing the mutarotation at 20° C reveals that the rapid reaction, $m_2$, is responsible for a change of 22.9°, whereas the slow reaction, $m_1$, is responsible for a change of 8.3°. Obviously, the rapid reaction comprises a large part of the mutarotation, in which respect the mutarotation of α-$d$-β-mannoheptose resembles the mutarotations of the structurally related sugars, α-$d$-talose [3] and α-$d$-α-guloheptose [7].

TABLE 1.—*Mutarotation of α-d-β-mannoheptose in distilled water* [1]

0.9981 g in 25 ml at 20.0° C read in a 4-dm tube
$$°S=+6.71+3.85×10^{-.0141t}+10.58×10^{-.0916t}$$

$$[α]_D^{20}=+14.5+8.3×10^{-.0141t}+22.9×10^{-.0916t}$$

Initial rotation, $[α]_D^{20}=+45.7$

Equilibrium rotation, $[α]_D^{20}=+14.5$

| Time | Observed reading | $(k_1+k_2)×10^3$ | $m_1×10^3$ | Deviation | $m_2×10^3$ |
|---|---|---|---|---|---|
| *Minutes* | °S | | | | |
| 2.55 | +16.44 | ---------- | ---------- | 6.18 | ---------- |
| 5.16 | +13.55 | 58.6 | ---------- | 3.58 | 90.8 |
| 7.57 | +11.88 | 54.7 | ---------- | 2.16 | 90.9 |
| 9.97 | +10.79 | 50.9 | ---------- | 1.29 | 91.7 |
| 13.20 | +9.85 | 46.1 | ---------- | 0.63 | 93.1 |
| 19.89 | +8.86 | 37.8 | ---------- | .13 | (96.7) |
| 30.10 | +8.16 | 30.0 | ---------- | ---------- | ---------- |
| 41.00 | +7.74 | 25.4 | 13.7 | ---------- | ---------- |
| 50.49 | +7.44 | 23.5 | 14.6 | ---------- | ---------- |
| 60.50 | +7.25 | 21.7 | 14.1 | ---------- | ---------- |
| 75.06 | +7.05 | 20.1 | 14.0 | ---------- | ---------- |
| 93.09 | +6.92 | 18.4 | (13.3) | ---------- | ---------- |
| ∞ | +6.71 | ---------- | ---------- | ---------- | ---------- |
| Average | ---------- | ---------- | 14.1 | ---------- | 91.6 |

1.013 g in 25.3 ml at 0.1° C in a 4-dm tube
$$°S=+7.65+4.32×10^{-.00181t}+8.95×10^{-.0159t}$$

$$[α]_D^{0.1}=+16.5+9.3×10^{-.00181t}+19.3×10^{-.0159t}$$

Initial rotation, $[α]_D^{0.1}=+45.1$

Equilibrium rotation, $[α]_D^{0.1}=+16.5$

| Time | Observed reading | $(k_1+k_2)×10^3$ | $m_1×10^3$ | Deviation | $m_2×10^3$ |
|---|---|---|---|---|---|
| 3.96 | +19.64 | ---------- | ---------- | 7.74 | ---------- |
| 8.99 | +18.21 | 10.95 | ---------- | 6.40 | 16.4 |
| 14.61 | +16.93 | 10.45 | ---------- | 5.21 | 16.1 |
| 19.73 | +15.99 | 9.99 | ---------- | 4.36 | 15.8 |
| 24.73 | +15.21 | 9.64 | ---------- | 3.66 | 15.7 |
| 29.81 | +14.52 | 9.35 | ---------- | 3.05 | 15.6 |
| 39.96 | +13.41 | 8.84 | ---------- | 2.10 | 15.7 |
| 50.04 | +12.62 | 8.30 | ---------- | 1.46 | 15.7 |
| 60.11 | +12.00 | 7.84 | ---------- | 0.99 | 15.9 |
| 75.21 | +11.37 | 7.13 | ---------- | .56 | 16.0 |
| 90.64 | +10.91 | 6.53 | ---------- | .30 | 16.3 |
| 147.25 | +9.99 | 4.95 | ---------- | ---------- | ---------- |
| 209.89 | +9.45 | 4.00 | 1.82 | ---------- | ---------- |
| 265.39 | +9.09 | 3.52 | 1.78 | ---------- | ---------- |
| 339.57 | +8.69 | 3.16 | 1.83 | ---------- | ---------- |
| ∞ | +7.65 | ---------- | ---------- | ---------- | ---------- |
| Average | ---------- | ---------- | 1.81 | ---------- | 15.9 |

[1] The equations and values for the mutarotation constants, $m_1$ and $m_2$, were determined by the method described by Isbell and Pigman, J. Research NBS 18, 156 (1937) RP969. The distilled water contained carbon dioxide and read pH=5.4.

Previous measurements with various sugars have revealed that the temperature coefficients for the interconversion of the alpha and beta

pyranoses are consistently larger than the temperature coefficients for the rapid reactions involving the labile modifications.[2] This is evidence that the heat of activation, $Q$, for the alpha-beta pyranose interconversion is larger than that characteristic of the rapid reaction. Application of the Arrhenius equation [3] to the velocity constants, $m_1$ and $m_2$, of $\alpha$-$d$-$\beta$-mannoheptose gives for the heat of activation, $Q$, values of 16,408 and 13,200. These values correspond to temperature coefficients [4] of 2.46 and 2.15, respectively. The value of $Q$ from $m_1$ is close to the values of $Q$ obtained from the constants representing the interconversion of the alpha and beta modifications of glucose (17,200), mannose (16,900), xylose (16,800), and other pyranoses. The value obtained from $m_2$ is close to the values obtained from the rapid mutarotations of arabinose (14,700), ribose (11,700), galactose (14,100) talose (12,800), and $d$-$\alpha$-guloheptose (12,610). Since the value obtained from $m_1$ is 16,408, while that from $m_2$ is only 13,200, the results are in accord with those obtained for other sugars and support the generalization that the heat of activation for the rapid reaction is less than the heat of activation for the slow reaction.

The effect of temperature on the equilibrium state is illustrated further by the thermal mutarotation given in table 2. After a solution of $d$-$\beta$-mannoheptose is cooled, the optical rotation increases in the dextro direction at a rate comparable to that of the rapid reaction ($m_2$). This increase in optical rotation which comprises the larger part of the mutarotation shows that the change in temperature causes a disturbance in the equilibrium proportions of the labile constituents. This initial equilibrium disturbance is followed by a readjustment of the proportions of the normal alpha and beta isomers which causes a small decrease in the optical rotation. The large alteration in the equilibrium proportions of the labile modifications with temperature, as shown by the thermal mutarotation, shows that the rapid reaction involves a change in energy. This observation is in accord with the generalization that the rapid reactions involving the labile modifications result in the liberation or absorption of heat, whereas the heat of reaction for the interconversion of the alpha and beta pyranoses is small. In this respect the large alteration in the equilibrium proportions of levulose at various temperatures might appear exceptional. It seems probable, however, that the mutarotation of levulose is like the rapid mutarotation reaction of galactose and differs from a normal alpha-beta reversible interconversion.

The mutarotations of $\alpha$-$d$-$\beta$-mannoheptose, $\alpha$-$d$-$\alpha$-guloheptose, and $\alpha$-$d$-talose resemble one another in striking manner and therefore support the generalization that the configurations of the carbon atoms comprising the pyranose ring largely determine the proportions of the various stereomeric modifications in the equilibrium solutions and the rates at which these constituents change from one to another. This similarity, which is obvious by comparing the structural formulas, is responsible in large measure for the similarity in the chemical properties of the structurally related sugars, which will be considered in the next section of this paper.

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[2] Page 165 of [3] and page 643 of [7].

[3] $2.3026 \log \dfrac{k_1}{k_2} = \dfrac{Q}{1.9864}\left(\dfrac{1}{T_2} - \dfrac{1}{T_1}\right)$.

[4] The temperature coefficient is the ratio of the rate at 35° C to that at 25° C and its logarithm equals $Q/42{,}011$.

TABLE 2.—*Mutarotation of a 3.3-percent aqueous solution of d-β-mannoheptose after cooling from 25° C to 0.1° C.*

$$°S = +0.21 \times 10^{-.0018t} - 1.34 \times 10^{-.015t} + 6.29$$

| Time | Saccharim-eterreading | Deviation | $m_2 \times 10^3$ |
|---|---|---|---|
| *Minutes* | °S | | |
| 3. 57 | +5. 31 | −1. 18 | |
| 6. 90 | +5. 47 | −1. 02 | 19. 0 |
| 9. 99 | +5. 55 | −0. 94 | 15. 4 |
| 15. 17 | +5. 68 | −. 80 | 14. 6 |
| 19. 96 | +5. 79 | −. 69 | 14. 2 |
| 30. 30 | +5. 96 | −. 51 | 13. 6 |
| 40. 22 | +6. 10 | −. 36 | 14. 1 |
| 49. 95 | +6. 18 | −. 28 | 14. 3 |
| 60. 05 | +6. 27 | −. 18 | 14. 5 |
| 151. 26 | +6. 40 | | |
| ∞ | +6. 29 | | |
| Average | | | 15. 0 |

## II. BROMINE OXIDATION MEASUREMENTS WITH d-α-GULOHEPTOSE AND d-β-MANNOHEPTOSE

Bromine oxidation measurements [3, 5, 12] made with many sugars, under controlled conditions in which the change of one modification to another is slow, have shown that the alpha and beta aldopyranoses are oxidized at widely different rates, and that the rates of oxidation can be correlated with the configuration of the first carbon in relation to the ring oxygen [11]. The aldose sugars (beta) in which the hydrogen of the first carbon is represented, in the conventional manner, as *cis* to the ring oxygen are oxidized more rapidly than the sugars (alpha) in which the hydrogen of the first carbon is represented as *trans* to the ring oxygen. The results given in tables 3 and 4 reveal that the rates of oxidation for the alpha and beta modifications of d-α-guloheptose and d-β-mannoheptose are in complete agreement with the correlation between the configuration of the first carbon and its rate of oxidation. As might be anticipated from their structures, α-d-β-mannoheptose (III) and α-d-α-guloheptose (IV) are oxidized at relatively slow rates. The equilibrium solutions of these sugars contain more rapidly oxidizable modifications, which are probably the corresponding beta sugars. The rates of reaction for α-d-β-mannoheptose (0.111) and for α-d-α-guloheptose (0.094) are comparable to the rate (0.078) previously found for α-d-talose. The more easily oxidizable substance in the solution of d-β-mannoheptose is oxidized 10 times as fast as the freshly dissolved alpha modification, whereas the more reactive substance in the solution of d-α-guloheptose is oxidized 13.6 times as fast as the freshly dissolved alpha form. Presumably the easily oxidizable fractions are largely the beta pyranose modifications, in which respect it is noted that β-d-talose is oxidized about 10 times as rapidly as α-d-talose and that the equilibrium solution of d-talose contains approximately 44 percent of the rapidly oxidizable fraction, whereas the equilibrium solution of d-β-mannoheptose contains 38 percent and that of d-α-guloheptose 33 percent of the rapidly oxidizable material.

TABLE 3.—*Bromine oxidations of freshly dissolved crystalline sugars at 0°C*[1]

| Time after beginning oxidation | Unoxidized sugar | Averages for oxidation period | | | Velocity constants $ak = \frac{1}{t} \log \frac{A}{A-X}$ | |
|---|---|---|---|---|---|---|
| | | Bromide (Br) | Bromine (Br$_2$) | Free bromine (a) | $ak \times 10^3$ | $k \times 10^3$ |

$\alpha$-d-$\beta$-MANNOHEPTOSE MONOHYDRATE

| Minutes | mg/ml | Moles/liter | Moles/liter | Moles/liter | | |
|---|---|---|---|---|---|---|
| 0.5 | 11.50 | 0.360 | 0.380 | 0.093 | ----------- | ----------- |
| 7.5 | 9.82 | .368 | .372 | .087 | 9.80 | 113 |
| 15.0 | 8.36 | .374 | .366 | .084 | 9.55 | 114 |
| 30.0 | 6.42 | .384 | .356 | .078 | 8.58 | 110 |
| 60.0 | 4.14 | .397 | .343 | .070 | 7.46 | 107 |
| Average | | | | | | 111 |

$\alpha$-d-$\alpha$-GULOHEPTOSE

| | | | | | | |
|---|---|---|---|---|---|---|
| 0.5 | 10.29 | 0.360 | 0.381 | 0.094 | ----------- | ----------- |
| 7.5 | 8.87 | .367 | .374 | .088 | 9.21 | 105 |
| 15.0 | 7.78 | .373 | .368 | .085 | 8.37 | 98 |
| 30.0 | 6.42 | .380 | .361 | .080 | 6.95 | 87 |
| 60.0 | 4.31 | .393 | .348 | .073 | 6.35 | 87 |
| Average | | | | | | 94.2 |

[1] Samples of the crystalline sugar (0.01 mole) with 1.2 g of barium carbonate were placed in 100-ml long-neck flasks and cooled to 0°C in an ice bath. To each flask 20 ml of a cold solution containing 60 g of Ba(Br)$_2$.2H$_2$O, 20 ml of bromine, and 4 ml of $N$ hydrobromic acid per liter were added and the mixture was shaken continuously. After definite time intervals the oxidation was stopped by the addition of 5 ml of linseed oil dissolved in 10 ml of benzene, and the reducing sugar in the aqueous solution was determined by the use of alkaline copper sulphate in the usual manner. The bromine (Br$_2$) in the oxidizing solution was determined by thiosulphate titration, and the concentration of the oxidant (free bromine) and the velocity constants were calculated as described on page 168 of [3].

TABLE 4.—*Bromine oxidations at 0° C in aqueous sugar solutions in equilibrium at the beginning of the oxidation* [1]

| Time after mixing solutions | Unoxidized sugar | | | Averages for the oxidation period | | | Velocity constants | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Total | More reactive fraction | Less reactive fraction | Bromide (Br) | Bromine (Br$_2$) | Free bromine (a) | More reactive fraction | | Less reactive fraction | |
| | | | | | | | $ak_B \times 10^3$ | $k_B \times 10^3$ | $ak_A \times 10^3$ | $k_A \times 10^3$ |

d-$\beta$-MANNOHEPTOSE (HYDRATE)

| Minutes | mg/ml | mg/ml | mg/ml | Moles/liter | Moles/liter | Moles/liter | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 11.88 | 4.44 | 7.44 | ----------- | ----------- | ----------- | ------- | ------- | ------- | ------- |
| .5 | 11.30 | 3.95 | 7.35 | 0.360 | 0.386 | 0.095 | ------- | ------- | ------- | ------- |
| 7.5 | 7.32 | 0.95 | 6.37 | .379 | .367 | .083 | 88.4 | 1,065 | ------- | ------- |
| 15.0 | 5.58 | .0 | 5.58 | .388 | .358 | .077 | ------- | ------- | ------- | ------- |
| 15.0 | 5.58 | ----------- | 5.58 | | | | | | | |
| 30.0 | 4.40 | ----------- | 4.40 | .417 | .331 | .062 | ------- | ------- | 6.87 | 111 |
| 60.0 | 3.00 | ----------- | 3.00 | .423 | .324 | .059 | ------- | ------- | 5.99 | 102 |
| Average | | | | | | | | 1,065 | ------- | 107 |

[1] The oxidations were conducted like the oxidations of the freshly dissolved crystalline sugars, except that a solution of the sugar in 10 ml of water was mixed with 10 ml of the oxidation solution adjusted to give the same concentrations of the reactants as those used for the oxidations reported in table 3. The proportions of the more reactive and less reactive fractions were calculated as described on page 177 of [3].

TABLE 4.—*Bromine oxidations at 0° C in aqueous sugar solutions in equilibrium at the beginning of the oxidation*—Continued

| Time after mixing solutions | Unoxidized sugar | | | Averages for the oxidation period | | | Velocity constants | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Total | More reactive fraction | Less reactive fraction | Bromide (Br) | Bromine (Br₂) | Free bromine (a) | More reactive fraction | | Less reactive fraction | |
| | | | | | | | $ak_B\times10^3$ | $k_B\times10^3$ | $ak_A\times10^3$ | $k_A\times10^3$ |
| *d*-α-GULOHEPTOSE | | | | | | | | | | |
| *Minutes* | *mg/ml* | *mg/ml* | *mg/ml* | *Moles/ liter* | *Moles/ liter* | *Moles/ liter* | | | | |
| 0 | 11.98 | 4.05 | 7.93 | --------- | --------- | --------- | ------- | ------- | ------- | ------- |
| .5 | 11.37 | 3.52 | 7.85 | 0.360 | 0.380 | 0.093 | ------- | ------- | ------- | ------- |
| 10.0 | 7.06 | 0.43 | 6.63 | .381 | .359 | .080 | ------- | ------- | ------- | ------- |
| 15.0 | 6.15 | --------- | 6.15 | .386 | .354 | .086 | 104.3 | 1,304 | ------- | ------- |
| 15.0 | 6.15 | --------- | 6.15 | | | | | | | |
| 30.0 | 4.95 | --------- | 4.95 | .412 | .328 | .062 | --------- | --------- | 6.29 | 101 |
| 60.0 | 3.56 | --------- | 3.56 | .421 | .321 | .058 | --------- | --------- | 5.28 | 91 |
| Average | --------- | --------- | --------- | --------- | --------- | --------- | --------- | 1,304 | --------- | 96 |

# III. EXPERIMENTAL DETAILS

## 1. SEPARATION OF *d*-α-MANNOHEPTONIC AND *d*-β-MANNOHEPTONIC ACIDS

The nitriles of *d*-α- and *d*-β-mannoheptonic acids were formed in the usual manner by treating 600 g of mannose in a 10-percent aqueous solution with equivalent quantities of sodium cyanide and calcium chloride [13]. Hydrolysis of the product with an excess of lime gave a precipitate of basic calcium salts which was collected on a filter and washed with limewater. These salts were decomposed by carbonation in a large volume of hot water to give the normal calcium salts. The aqueous solution was evaporated and about 250 g of crystalline calcium *d*-α-mannoheptonate was separated. The residual liquor contained a small quantity of calcium *d*-β-mannoheptonate mixed with calcium *d*-α-mannoheptonate. To separate the constituent acids, the calcium was removed with aqueous sulphuric acid and the resulting heptonic acids were converted to difficultly soluble normal lead salts by reaction with an equivalent quantity of lead oxide. The resulting lead salts were dissolved in hot water and fractionally crystallized in the following manner. The hot aqueous solution of the salts was seeded with lead *d*-α-mannoheptonate and allowed to cool slowly. Considerable lead *d*-α-mannoheptonate separated from the solution in slender needle-like prisms before lead *d*-β-mannoheptonate began to crystallize in rhomb-shaped plates. The needle-like crystals were collected in one fraction and the rhombic plates in another. By repeated fractionation and recrystallization about 50 g of pure lead *d*-β-mannoheptonate and 100 g of lead *d*-α-mannoheptonate were separated from the preparation which began with 600 g of mannose.

## 2. *d*-β-MANNOHEPTONIC ACID

The preparation of this acid in the crystalline state was reported previously by V. Ettel [14]. It resembles talonic acid in that it is relatively difficultly soluble and crystallizes readily. The acid ob-

tained from lead *d*-$\beta$-mannoheptonate by treatment with sulphuric acid was recrystallized from aqueous isopropyl alcohol. The product so obtained is dextrorotatory and exhibits the mutrarotation given in table 5. The changes in optical rotation indicate that a dextrorotatory delta lactone and a levorotatory gamma lactone are formed and that the delta lactone is formed more rapidly than the gamma lactone.

TABLE 5.—*Mutarotation of d-$\beta$-mannoheptonic acid in 4-percent aqueous solution*

| Time | $[\alpha]_D^{20}$ | Time | $[\alpha]_D^{20}$ |
|---|---|---|---|
| *Minutes* | | *Minutes* | |
| 5 | +4.0 | 240 | +6.1 |
| 20 | +5.4 | 1,510 | −3.2 |
| 30 | +5.7 | 2,885 | −8.8 |
| 60 | +6.2 | 5,765 | −17.1 |
| 123 | +6.7 | 30 days | −25.0 |

*d-$\beta$-Mannoheptonic $\gamma$-lactone* was prepared by evaporating and heating an aqueous solution of *d*-$\beta$-mannoheptonic acid in the presence of a few drops of hydrochloric acid. The resulting thick sirup was dissolved in ethyl alcohol and allowed to stand in a desiccator over sodium hydroxide. Crystallization occurred in the course of several weeks. The crude product was recrystallized from hot absolute ethyl alcohol. The new substance separates in concentric clusters of long, thin crystals which melt at 130° C and give $[\alpha]_D^{20} = -35.7$ The product corresponds to the following analysis: Calculated for $C_7H_{12}O_7$: C, 40.39; H, 5.81. Found: C, 40.45; H, 5.82. The direction of the optical rotation and the rate of hydrolysis as indicated by the slow mutarotation given in table 6 show that the product is the gamma rather than the delta lactone. It is slightly sweet and requires one equivalent of alkali for saponification.

TABLE 6.—*Mutarotation of d-$\beta$-mannoheptonic $\gamma$-lactone in water at 20° C*

[4.0152 g per 100 ml in a 4-dm tube]

| Time | Observed rotation | $[\alpha]_D^{20}$ | Time | Observed rotation | $[\alpha]_D^{20}$ |
|---|---|---|---|---|---|
| *Minutes* | °S | | *Days* | °S | |
| 4.0 | −8.29 | −35.7 | 1 | −8.10 | −34.9 |
| 10.1 | −8.27 | −35.7 | 3 | −7.91 | −34.1 |
| 20.1 | −8.29 | −35.7 | 31 | −6.64 | −28.6 |
| 372.0 | −8.25 | −35.6 | 105 | −6.48 | −27.9 |

*Potassium d-$\beta$-mannoheptonate* was prepared in aqueous alcoholic solution from equivalent quantities of *d*-$\beta$-mannoheptonic acid and potassium hydroxide. The salt gives triangular crystals which form readily and can be used for purification. After drying at 40° C in vacuo the new salt gives $[\alpha]_D^{20} = +0.3$ (in 4-percent aqueous solution) and corresponds to the following analysis: Calculated for $KC_7H_{13}O_8$: C, 31.81; H, 4.96; K, 14.79. Found: C, 32.06; H, 4.97; K, 14.6.

*Lead d-$\beta$-mannoheptonate* was separated from the lead-salt mixture which resulted from the cyanhydrin synthesis. The salt crystallizes in laminated clusters of rhomb-shaped plates which are slightly soluble in hot water and very difficultly soluble in cold water; at 20° C

0.255 g dissolves in 100 ml of water. One gram of the crystals dissolved in 100 ml of hot water and then cooled to 20° C gives $[\alpha]_D^{20} =$ +2.16. The salt is anhydrous as shown by the following analysis: Calculated for Pb $(C_7H_{13}O_8)_2$: C, 25.57; H, 3.98; Pb, 31.51. Found: C, 25.7; H, 3.9; Pb, 31.5.

### 3. α-d-β-MANNOHEPTOSE HYDRATE

Fifty grams of d-β-mannoheptonic γ-lactone was reduced in the usual manner [15] with 1,500 g of 3-percent sodium amalgam, after which an analysis showed the presence of 35 g of sugar. Following the removal of the sodium sulphate and excess d-β-mannoheptonate by alcohol precipitation, the reduced solution was concentrated to a thick sirup, which was dissolved in aqueous isopropyl alcohol. After several days, crystals of α-d-β-mannoheptose monohydrate formed. The crystals were separated and recrystallized from aqueous isopropyl alcohol. The new sugar separates in long thin wedge-shaped crystals which melt at 83° C and correspond to the following analysis: Calculated for $C_7H_{14}O_7 \cdot H_2O$: C, 36.84; H, 7.07. Found: C, 36.9; H, 6.9. The sugar exhibits the complex mutarotation given in table 1. The final specific rotation of the hydrated sugar at 20° C is +14.5 and the initial rotation is +45.7.

The relative copper-reducing value measured with Benedict's solution, using 4 minutes to bring to boiling and boiling 6 minutes, and based on the weight of the anhydrous sugar, is 83 percent of that of glucose. This value is the same as that obtained for the epimeric sugar, d-α-mannoheptose, and is close to the values obtained for the structurally related sugars, d-α-guloheptose and d-β-guloheptose.

A crystalline modification of d-β-mannoheptose which melts at 140° C and gives $[\alpha]_D = 7.6$ was described by Ettel [14] but unfortunately the physical and chemical properties are not reported in sufficient detail to permit classification of the sugar. Since the optical rotation differs markedly from that of α-d-β-mannoheptose, Ettel's product represents either another modification of the sugar or an entirely different substance.

### 4. d-α-MANNOHEPTONIC ACID

This acid has been investigated by previous workers [9,10,15] who prepared the crystalline acid, its gamma lactone, and the sodium, ammonium, calcium, barium, strontium, and cadmium salts. The lead and potassium salts are reported in this paper. *Lead d-α-mannoheptonate* crystallizes in slender prisms which are slightly soluble in hot water and difficultly soluble in cold water; at 20° C 0.046 g dissolves in 100 ml of water. The air-dried salt contains one molecule of water of crystallization and corresponds to the following analysis: Calculated for $Pb(C_7H_{13}O_8)_2 \cdot H_2O$: C, 24.89; H, 4.18; Pb, 30.67. Found: C, 25.2; H, 4.3; Pb, 30.65. *Potassium d-α-mannoheptonate* crystallizes in needles which are very soluble in water and give $[\alpha]_D^{20} = +5.4$ in 4-percent aqueous solution. The salt corresponds to the following analysis: Calculated for $KC_7H_{13}O_8$: C, 31.81; H, 4.96; K, 14.79. Found: C, 31.64; H, 5.08; K, 14.7.

### 5. α-d-α-MANNOHEPTOSE HYDRATE

Crude d-α-mannoheptose prepared by the method of Fischer and Passmore [15] was recrystallized in the following manner: An aqueous

solution containing 50 g of sugar was dehydrated by evaporation to a thick sirup followed by the addition of absolute alcohol and a second evaporation. The residue was taken up in absolute alcohol and brought to crystallization. The product which was probably the anhydrous alpha form melted at 140° C. On recrystallization from cold aqueous alcohol this product was converted to a monohydrate which melts at 107° C and gave the following analysis: Calculated for $C_7H_{14}O_7.H_2O$: C, 36.84; H, 7.07. Found: C, 36.96; H, 7.26. As reported on page 517 of reference [5], the mutarotation at 20° C is represented by the following equation: $[α]_D^{20} = +3.4 \times 10^{-.0485t} + 51.9 \times 10^{-.00391t} + 64.7$.

### 6. β-d-α-MANNOHEPTOSE HYDRATE

This sugar was prepared in the following manner: Sixty grams of crude *d-α*-mannoheptose was dissolved in 40 ml of hot water. After the lapse of sufficient time for the sugar to reach equilibrium the solution was filtered and mixed with 200 ml of acetic acid. Upon cooling, rectangular prismatic crystals of *β-d-α*-mannoheptose hydrate separated. The new sugar melts at 104° C and corresponds to the following analysis: Calculated for $C_7H_{14}O_7.H_2O$: C, 36.84; H, 7.07. Found: C, 37.04; H, 7.19. The final specific rotation at 20° C is +64.5 and the initial rotation is +42.3. The mutarotation at 20° C and bromine-oxidation measurements were reported previously [5].

## IV. GENERAL SUMMARY

By treating mannose with sodium cyanide followed by lime, basic calcium salts of the epimeric mannoheptonic acids were precipitated. Decomposition of the basic salts by treatment with carbon dioxide gave a mixture of the normal salts from which crystalline calcium *d-α*-mannoheptonate was separated. The salts in the mother liquor were converted to lead salts which were separated by fractional crystallization. *Lead d-α-mannoheptonate*, $Pb(C_7H_{13}O_8)_2.H_2O$, forms slender prisms which are very difficultly soluble in water. *Potassium d-α-mannoheptonate*, $KC_7H_{13}O_8$, crystallizes in needles which are very soluble in water and gives $[α]_D^{20} = +5.4$ in 4-percent aqueous solution. *Lead d-β-mannoheptonate*, $Pb(C_7H_{13}O_8)_2$, crystallizes in rhomb-shaped plates which give $[α]_D^{20} = +2.16$ in 1-percent aqueous solution. *Potassium d-β-mannoheptonate*, $KC_7H_{13}O_8$, crystallizes in triangular plates which give $[α]_D^{20} = +0.3$. *d-β-Mannoheptonic* γ-lactone, $C_7H_{12}O_7$, forms long thin crystals which melt at 130°C and give $[α]_D^{20} = -35.7$. Reduction of *d-β*-mannoheptonic γ-lactone gives *α-d-β*-mannoheptose hydrate, $C_7H_{14}O_7.H_2O$, which melts at 83°C and gives $[α]_D^{20} = +45.7$ with mutarotation. The mutarotations at 20° and 0.1°C are represented by the following equations:

$$[α]_D^{20} = +14.5 + 8.3 \times 10^{-.0141t} + 22.9 \times 10^{-.0916t}$$

$$[α]_D^{1} = +16.5 + 9.3 \times 10^{-.00181t} + 19.3 \times 10^{-.0159t}$$

The mutarotation, which resembles that of *α-d*-talose, reveals a fast and a slow reaction. The proportions of the constituents involved in the fast reaction vary with temperature so that the equilibrium rotation also varies with temperature. The temperature coefficient for the rapid reaction corresponds with the coefficients previously obtained for the rapid reactions found in other complex mutarota-

tions and for the mutarotation of levulose, whereas the temperature coefficient for the slow reaction corresponds with the values previously obtained for the reversible interconversion of the alpha and beta pyranoses.

Bromine oxidations of $\alpha$-$d$-$\alpha$-guloheptose and of $\alpha$-$d$-$\beta$-mannoheptose take place slowly at rates comparable to the oxidation of $\alpha$-$d$-talose. The oxidations of equilibrium solutions of the sugars proceed rapidly until a portion of the sugar is used up and then more slowly as the remaining sugar continues to be oxidized. The results show that equilibrium solution of $d$-$\alpha$-guloheptose contains 33 percent of some substance which is oxidized about 13.6 times as fast as $\alpha$-$d$-$\alpha$-guloheptose; and that the equilibrium solution of $d$-$\beta$-mannoheptose contains 38 percent of some substance which is oxidized about 10 times as fast as $\alpha$-$d$-$\beta$-mannoheptose

The marked resemblance of $\alpha$-$d$-$\alpha$-guloheptose and $\alpha$-$d$-$\beta$-mannoheptose to $\alpha$-$d$-talose and other alpha sugars illustrates clearly the advantages of classifying the alpha and beta sugars according to the configuration of the first carbon in relation to the pyranose ring. The results furnish additional evidence to show that the configurations of the five carbon atoms comprising the pyranose ring determine in large measure the peculiarities found in the mutarotations of sugars which contain the galactose, talose, and idose structures.

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