

TWO ISOMERIC CRYSTALLINE COMPOUNDS OF D-MANNOSE WITH CALCIUM CHLORIDE¹

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ABSTRACT

A crystalline double compound of mannose and calcium chloride having the formula $C_6H_{12}O_6 \cdot CaCl_2 \cdot 4H_2O$ has been prepared. The compound showed an extremely rapid initial rate of mutarotation from about $[\alpha]_D = -30$ to a positive maximum followed by a slow decrease to a constant value. The sugar constituent of this compound is a new form of mannose, and application of Hudson's rule indicates that it is the beta form corresponding in ring structure with α -mannose of $[\alpha]_D = +30$. A second isomer having the formula $C_6H_{12}O_6 \cdot CaCl_2 \cdot 2H_2O$ was obtained by evaporating an alcoholic solution of the above compound on a water bath. Its initial and final specific rotation and course of mutarotation indicate that it is a double compound of the well-known β -mannose of $[\alpha]_D = -17$ with calcium chloride. The second compound, but not the first, could be acetylated to give a good yield of β -mannose penta-acetate.

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I. INTRODUCTION

Crystalline compounds of the sugars with inorganic salts, a number of which are known,³ have hitherto been of little interest in the advancement of our knowledge concerning the structures of the sugars. However, the important position which mannose holds in relation to structural studies makes the preparation and study of any

¹ The writer has arranged with Dr. C. S. Hudson that the present communication be included as No. 19 in the series entitled "Relations Between Rotatory Power and Structure in the Sugar Group."

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³ v. Lippmann, *Chemie der Zuckerarten*, 3d ed., pp. 549 and 884.

of its compounds or derivatives of unusual interest. Dr. C. S. Hudson informed the author that he had observed the formation of crystals in a solution of mannose and calcium chloride and suggested that the writer prepare and investigate the compound whose existence in a crystalline state had been thus indicated. This proposal led to far more interesting results than had been anticipated. The double compound of mannose and calcium chloride which was obtained showed mutarotation, but the course of the mutarotation was of an

Initial Stages in the Mutarotation of Mannose - CaCl_2 Compounds.

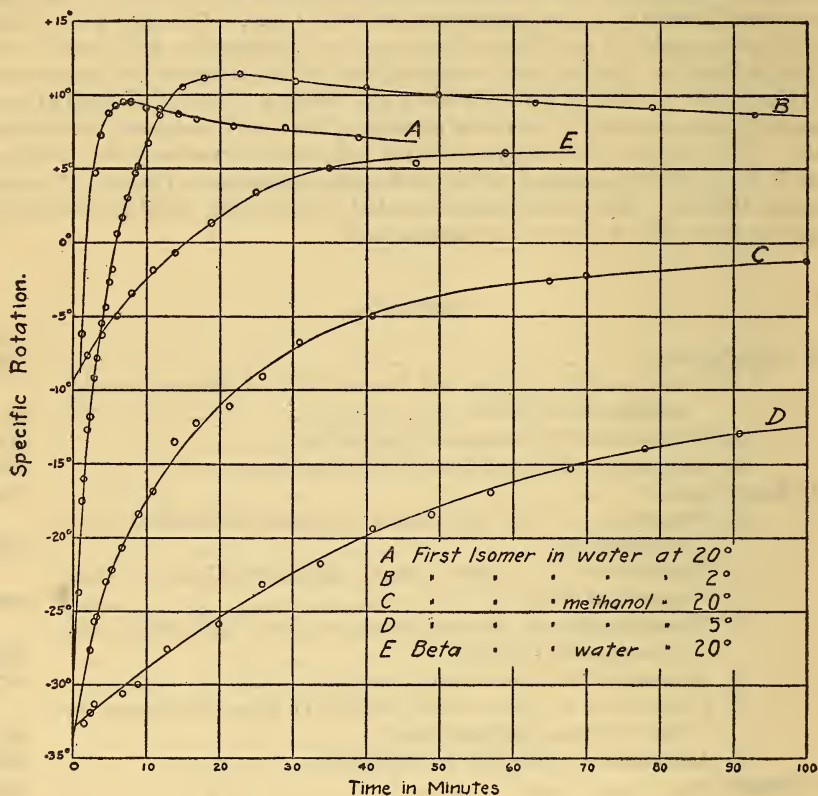


FIGURE 1

unusual character differing from that of any known sugar. Secondly, by evaporating an alcoholic solution on a water bath the compound was transformed into a second modification, isomeric with the original. Subsequently it was found that either isomer could be made at will from mannose and calcium chloride, or even directly from hydrolyzed vegetable ivory, depending upon the solvent and the temperature at which crystallization took place. The first isomer crystallizes from a concentrated aqueous solution of mannose and calcium chloride at room temperature, while the second crystallizes

from an alcoholic solution during evaporation on a boiling water bath. The isomer crystallizing from water at room temperature showed a calcium and chlorine content, loss of weight on drying, and specific rotation at equilibrium corresponding to the formula $C_6H_{12}O_6 \cdot CaCl_2 \cdot 4H_2O$. As its initial specific rotation and course of mutarotation, in the early stages at least, do not permit a correlation with either of the known forms of mannose, it will subsequently simply be designated as the "first isomer." The second isomer, crystallizing from alcohol at the temperature of a boiling water bath, showed a calcium and chlorine content corresponding to the formula $C_6H_{12}O_6 \cdot CaCl_2 \cdot 2H_2O$. Its initial specific rotation and course of mutarotation correlate it with β -mannose of initial $[\alpha]_D = -17$; hence, it will be designated as β -mannose- $CaCl_2 \cdot 2H_2O$.

1. INITIAL SPECIFIC ROTATION AND MUTAROTATION OF THE FIRST ISOMER, MANNOSE- $CaCl_2 \cdot 4H_2O$

The first observations of the direction of the mutarotation of this compound indicated that the optically active component was α -mannose. However, an extrapolation of its mutarotation curve to zero time showed an initial specific rotation, calculated to a mannose basis, of $[\alpha]_D = +20$, whereas Levene⁴ had found $[\alpha]_D = +30$ for α -mannose. Subsequent attempts to obtain readings more quickly after making the solution gave the unexpected result that the anticipated normal mutarotation was preceded by an extremely rapid change from a negative value, far below even that of β -mannose, to a positive maximum. From this maximum the rotation fell at a rate comparable with that of α -mannose. At 20° in aqueous solution the initial change was too rapid to be accurately measured. At 2° it was still rapid, but could be followed with sufficient precision to permit a close estimation of the initial value. In Tables 1 and 2 are recorded the mutarotation data for this compound in aqueous solution at the temperatures of 20° and 2°, respectively. The curves *A* and *B* of the figure are plotted from these readings.

The later part of the mutarotation, decreasing from the maximum to an equilibrium value, appears to be unimolecular, and the constant found at 20°, $k_1 + k_2 = 0.025$, is of the same order of magnitude as that given by Hudson and Sawyer for β -mannose⁵ (0.019), and Levene⁶ has shown that α -mannose has the same rate of mutarotation as the beta form. Furthermore, if this portion of the curve is extrapolated to zero time the initial specific rotation thus estimated is not far removed from that of α -mannose. These facts constitute evidence that this stage of the mutarotation is the passing of α -mannose to the equilibrium state of mannose solutions.

⁴ J. Biol. Chem., 57, p. 329; 1923; 59, p. 129; 1924.

⁵ Hudson and Sawyer, J. Am. Chem. Soc., 39, p. 475; 1917.

⁶ See footnote 4.

TABLE 1.—Mutarotation of the first isomer, *d*-Mannose- $\text{CaCl}_2 \cdot 4\text{H}_2\text{O}$, in water[1.7437 g compound in 25 ml solution. Tube length, 2 dm. $T=20^\circ \pm 0.5^\circ$]

Time after making solution (minutes)	$[\alpha]_D^{20}$ of compound	Time	$k_1+k_2=\frac{1}{T} \log \frac{r_0-r_\infty}{r-r_\infty}$
1½	-6.22		
3	+4.73		
4	+7.21		
5	+8.74		
6	+9.20		
7	+9.46		
8	+9.46	0	
10	+9.08	2	0.032
12	+8.95	4	.022
14½	+8.64	6½	.024
17	+8.33	9	.026
22	+7.80	12	.028
29	+7.71	21	.021
39	+7.09	31	.028
∞	+6.72		
Average			.025

TABLE 2

[2.856 g compound in 25 ml solution. Tube length, 2 dm. $T=2^\circ \pm 0.5^\circ$]

Time after making solution (minutes)	$[\alpha]_D^{20}$ of compound	Time	$k_1+k_2=\frac{1}{T} \log \frac{r_0-r_\infty}{r-r_\infty}$
¾	-23.68		
1¼	-17.60		
1½	-15.97		
2	-12.75		
2¼	-11.83		
3	-9.10		
3½	-7.89		
4	-5.46		
4½	-4.55		
5	-2.73		
5½	-1.82		
6	+1.61		
6¾	+1.82		
7½	+3.03		
8¼	+4.85		
9	+5.16		
10¼	+6.68		
12	+8.62		
15	+10.50		
18	+11.05		
23	+11.35	0	
30½	+10.93	7½	0.0052
40	+10.42	17	.0054
50	+10.00	27	.0053
63	+9.42	40	.0054
79	+9.10	56	.0048
93	+8.62	70	.0051
111	+7.71	88	[.0068]
130	+7.10	107	[.0085]
147	+6.68	124	[.0115]
∞	+6.50	∞	
Average			.0052

The initial rapid increase in rotation deserves special attention. The possibility exists that this change is due to a dissociation of the mannose calcium chloride compound. This explanation appears unlikely, however, because no change in polarization due to a slow dissociation of sugar and salt has been observed in other compounds of this nature.⁷ It appears to be more likely that this change is due to a rapid rearrangement of a new form of mannose to the alpha modification. If this is true, the initial specific rotation of this new form of mannose becomes of special interest. Extrapolation of the mutarotation curve *B* to zero time shows the initial specific rotation of this compound to be about -30 and hence for the mannose constituent $[\alpha]_D = -60$. Owing to the extreme rapidity of the early stages of the polarimetric change this figure is uncertain by several degrees.

The rate of the polarimetric change of the first isomer in methanol is much slower than in water; hence in this solvent the course of the change could be followed with more precision and the initial specific rotation determined with more accuracy. Curves *C* and *D* show the early stages of the mutarotation of this compound at the temperatures of 20° and 5° , respectively. Extrapolation of these curves to zero time gives the values for the initial specific rotation at these two temperatures as -34 and -33 , and, hence for the mannose constituent, $[\alpha]_D = -68.6$ and -66.5 . The final or equilibrium value for the specific rotation in this solvent was found to be $+4.0$ and $+4.8$, an average of $+4.4$, which calculated to a mannose basis equals $+8.9$.

C. S. Hudson⁸ has produced evidence from theoretical considerations based upon the principle of optical superposition that the two known crystalline forms of mannose are not a true alpha and beta pair having the same ring structure, but that the form with an initial specific rotation $+30$ is the alpha form of a 1, 5 ring structure, while the form showing $[\alpha]_D = -17$ is the beta form of a 1, 4 ring structure. He has further calculated the specific rotations to be expected for the missing members of these 2 ring structures and predicts the value for the β form of the 1, 5 ring structure to be -65° in water solution. The initial specific rotation of a new form of mannose, occurring as a crystalline double compound with CaCl_2 , is here shown to be not far from -60 in aqueous solution. The close agreement of this measured initial specific rotation of the new form of mannose with one of Hudson's calculated values, -65 , lends strong experimental support to his view that the well-known crystalline forms of mannose possess different ring structures, and as a corollary it follows that the mannose

⁷ The author has prepared in crystalline form calcium chloride compounds of arabinose, xylose, and fructose, and also the well-known compound of sodium chloride and glucose. Each of these substances shows a normal mutarotation comparable with that of the sugar constituent. The data will be published in a subsequent article.

⁸ C. S. Hudson, J. Am. Chem. Soc., 48, pp. 1425 and 1434. 1926.

constituent of the crystalline compound here described is the beta form of the sugar, corresponding in ring structure with alpha mannose of initial specific rotation $+30$. If this view is correct, the initial mutarotation must be explained as a rapid conversion of the beta form of one ring structure to the corresponding alpha form. The subsequent slower polarimetric change must then be considered to be an establishment of equilibrium between forms of different ring structures.

2. MUTAROTATION OF β -MANNOSE \cdot $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$

This compound, which was first obtained by concentrating an alcoholic solution of the first isomer on a boiling water bath, was characterized by a normal mutarotation in aqueous solution, as shown in Table 3 and by curve *E*. The mutarotation constant, $k_1 + k_2$, at 20° was found to be 0.024, a value essentially the same as the constant found for the latter stage of the mutarotation of the first isomer (0.025) and slightly higher than the value, 0.019, found for β -mannose⁹ at this temperature.¹⁰ The final specific rotation, $+6.73$, and the extrapolated initial specific rotation, -9.0 , correspond to $+12.2$ and -16.2 , respectively, for the mannose constituent. Thus, this isomer is apparently a compound of CaCl_2 with the well known β -mannose, of $[\alpha]_D = -17$.

3. ACETYLATION OF THE MANNOSE \cdot CaCl_2 COMPOUNDS

β -mannose \cdot $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ may be of utility in the synthesis of mannose derivatives because it can be directly acetylated to give a good yield of β -mannose pentaacetate. This may be of timely importance, for pure β -mannose has recently become difficult to obtain, at least in some laboratories. Levene¹¹ states that after having once prepared α -mannose subsequent recrystallizations of impure mannose from alcohol invariably gave this form. The author recrystallized at intervals several lots of impure mannose in the research laboratory of St. Andrews University. At first β -mannose was obtained, but unexpectedly one lot appeared as the alpha isomer, and thereafter the recrystallized product always consisted of this form. It is true that alpha mannose can be acetylated¹² to give alpha mannose pentaacetate, but the yield is not more than 20 per cent of the theoretical which is far from satisfactory. On the other hand, β -mannose or

⁹ Hudson and Sawyer, J. Am. Chem. Soc., 39, p. 475; 1917.

¹⁰ The difference between the value $k_1 + k_2 = 0.024$ found for β -mannose $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and the value $k_1 + k_2 = 0.019$ found by Hudson and Sawyer for β -mannose is too great to be attributed to experimental error. It is more likely that the rate of mutarotation of the mannose constituent of β -mannose \cdot $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ is accelerated by the presence of CaCl_2 in the solution. In confirmation a mutarotation experiment was made upon β -mannose dissolved in water and a second one upon β -mannose dissolved in a CaCl_2 solution of such strength that mannose and CaCl_2 were present in equimolecular proportions. Mannose in water alone showed $k_1 + k_2 = 0.020$, while in the CaCl_2 solution it showed $k_1 + k_2 = 0.025$.

¹¹ See footnote 4, p. 461.

¹² Levene, J. Biol. Chem., 57, p. 329; 1923.

β -mannose·CaCl₂·2H₂O readily yields on acetylation 60 per cent or more of the theory. The first isomer (mannose·CaCl₂·4H₂O) on acetylation with acetic anhydride and pyridine in an ice bath gave a sirupy gum which after long standing in an ice chest yielded only a few crystals of β -mannose pentaacetate.

TABLE 3.—Mutarotation of β -mannose·CaCl₂·2H₂O in water

[3.0146 g compound in 25 ml solution. Tube length 2 dm. $T=20^{\circ}\pm 0.5^{\circ}$]

Time after making solution (minutes)	$[\alpha]_D^{20}$ compound	Time	k_1+k_2
2	-7.70	0	
4	-6.33	2	0.022
6	-5.02	4	.025
8	-3.47	6	.025
11	-1.97	9	.024
14	-.68	12	.024
19	+1.20	17	.024
25	+3.30	23	.027
35	+4.93	33	.027
47	+5.35	45	.022
59	+5.97	57	.023
∞	+6.73	∞	
Average			.024

II. EXPERIMENTAL

1. PREPARATION OF THE FIRST ISOMER, MANNOSE·CaCl₂·4H₂O, FROM β -*d*-MANNOSE

Twenty grams of β -mannose and 32 g crystallized calcium chloride dihydrate were dissolved on a boiling water bath in 28 ml of water. On standing over night at room temperature the solution crystallized to a solid mass. This was thinned out with absolute alcohol, filtered on a Büchner funnel, and washed with absolute alcohol. Dried at 35°, the yield was 28 g. It was recrystallized by slow evaporation of a concentrated aqueous solution in a desiccator. A solution of 25 g of the compound in 25 ml of cold water was filtered through an asbestos mat followed by 10 ml of water. The filtrate was placed in a crystallizing dish in a vacuum desiccator over H₂SO₄. After two weeks this solution had crystallized to a solid mass. It was triturated with absolute alcohol, filtered on a suction filter, and washed with a further quantity of alcohol. Dried at 35°, the yield was about two-thirds of the original material. It was readily soluble in water, methanol, and hot absolute alcohol. It dissolved slowly in cold 95 per cent alcohol and in acetone. It melted at 101° to 102° corr.

Analysis.—Chlorine and calcium were determined directly in an aqueous solution of the compound. Moisture was determined by drying at 50 mm over P₂O₅, first for six hours at 76°, then for 19 hours at 100°. Four hours additional drying at 110° caused no further loss in weight.

1.0197 g gave 0.8080 g AgCl, hence Cl = 19.62 per cent.

1.0239 g gave 0.1615 g CaO, hence Ca = 11.16 per cent.

Loss on drying to constant weight equaled 19.10 per cent H₂O.

Observed $[\alpha]_D^{20} = +6.72$.

Calculated for mannose·CaCl₂·4H₂O, Cl = 19.56, Ca = 11.02, H₂O = 19.80, $[\alpha]_D^{20} = +6.94$.

2. PREPARATION OF THE FIRST ISOMER, MANNOSE·CaCl₂·4H₂O, FROM VEGETABLE IVORY

A mannose sirup was prepared essentially according to the methods of Hudson and Sawyer¹³ and Clark¹⁴ though with a somewhat simplified procedure. One hundred grams of vegetable ivory meal sifted through a 48-mesh sieve was slowly added to 125 g of 85 per cent H₂SO₄ and kneaded into a thick paste. After standing at room temperature for 24 hours the paste, which had by this time become liquid, was diluted to a volume of 2 liters and boiled under a reflux condenser for three hours, or until samples which were taken at one-half hour intervals showed a constant rotation. The dark red liquor was decolorized with 20 g of active carbon and neutralized to congo red paper by slowly adding freshly precipitated, well-washed barium carbonate to the boiling solution. The precipitated barium sulphate was separated by filtration with suction and well washed. The mixed filtrate and washings were acidified with 2 ml of 5 per cent H₂SO₄, and the slight precipitate thus produced was filtered off with the addition of a further 10 g of decolorizing carbon. This colorless liquor was evaporated to a sirup of about 90 per cent solids as determined with a refractometer. To this sirup, while still warm, there was added a solution of 95 g of calcium chloride dihydrate in 60 ml of water. The mixture was stirred to a uniform solution, which was then diluted with 135 ml of equal parts of absolute alcohol and acetone. Crystallization began almost immediately after cooling and seeding. After one night at room temperature and 24 hours in an ice chest the crystals were filtered off on a Büchner funnel and washed with 400 ml of equal parts of absolute alcohol and acetone. Dried at 35° there was obtained 114 g. The melting point, analysis, and polarization data identified this product with the first isomer, mannose·CaCl₂·4H₂O, as originally obtained from crystalline β-mannose.

3. TRANSFORMATION OF THE FIRST ISOMER, MANNOSE·CaCl₂·4H₂O INTO β-MANNOSE·CaCl₂·2H₂O

Ten grams of the first isomer was dissolved in 40 ml of hot absolute alcohol and the solution was placed on a boiling water bath. When the volume had been reduced by about one-quarter, crystals appeared.

¹³ See footnote 9, p. 464.

¹⁴ Clark, J. Biol. Chem., 51, p. 1; 1922.

By the time the volume had been further reduced to about one-half, the solution was well filled with crystals. It was then set aside at room temperature over night, filtered, and the crystals washed with absolute alcohol. Dried at 35° there was obtained 6½ g. The product was markedly different from the first isomer. The crystals were well defined, hard, triangular prisms. They were readily soluble in water and in methanol, only slightly so in cold 95 per cent alcohol, and practically insoluble in absolute alcohol. The substance melted at 159° to 160° (corr.) with decomposition.

Analysis.—Chlorine and calcium were determined directly in aqueous solution. The results agree with the percentages calculated for mannose·CaCl₂·2H₂O, but holding at 50 mm over P₂O₅ at 100° for five hours caused no loss in weight.

0.9799 g gave 0.8550 g AgCl, hence Cl = 21.59 per cent.

1.0168 g gave 0.1741 g CaO, hence Ca = 12.23 per cent.

Observed $[\alpha]_{20}^D = +6.73$.

Calculated for mannose·CaCl₂·2H₂O, Cl = 21.71; Ca = 12.23; $[\alpha]_{20}^D = +7.7$.¹⁵

4. REVERSAL OF THE ABOVE TRANSFORMATION

Ten grams of β -mannose·CaCl₂·2H₂O was dissolved in 150 ml boiling absolute alcohol. The solution was cooled and 1.1 ml of water was added, thus sufficient to make up the deficiency between a dihydrate and a tetrahydrate. The solution was evaporated at room temperature in a current of air to a thin sirup, then seeded with crystals of the first isomer and placed in a desiccator. After a few days the sirup had crystallized to a semisolid mass. After filtering, washing with absolute alcohol, and drying at 35° there was obtained 6 g. The melting point and course of mutarotation identified it as the first isomer.

5. PREPARATION OF β -MANNOSE·CaCl₂·2H₂O FROM β -MANNOSE OR DIRECTLY FROM VEGETABLE IVORY

This compound, though first prepared by transformation of the first isomer as described above, was subsequently obtained both from crystalline β -mannose and from hydrolyzed vegetable ivory. In the former case 20 g β -mannose with 24 g calcium chloride dihydrate was dissolved in 12 ml of hot water, the solution placed on a boiling water bath, and 80 ml of hot absolute alcohol slowly added. After remaining on the bath for one-half hour the solution was well filled with crystals. It was cooled slowly and kept at room temperature overnight. Filtered, washed with 95 per cent alcohol, and dried at 35° there was obtained 28 g.

¹⁵ The formula, mannose·CaCl₂·2H₂O is based upon the close agreement of the theoretical percentages of Ca and Cl with the values found. The fact that no loss of weight occurred on drying under the conditions stated does not exclude this formula. Three other compounds of this type, xylose, arabinose, and fructose calcium chlorides, also give percentages of Ca and Cl which correspond closely for hydrates, but do not lose weight on drying over P₂O₅ at 100° and 50 mm.

The same procedure of crystallizing from strong, hot alcohol was applied to the preparation of this compound directly from an impure mannose sirup. Ten grams of a thick sirup from hydrolyzed vegetable ivory prepared as described earlier was mixed with 10 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, previously dissolved in 3 ml of water, on a boiling water bath. Thirty-five milliliters of warm absolute alcohol was added, and the resulting solution kept on the bath until its volume had been reduced by about one-half, when a few crystals of β -mannose- $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ were added as seed. Crystallization of the characteristic triangular prisms was soon in evidence and progressed rapidly. After a further short period on the bath and 48 hours at room temperature the crystals were separated on a Büchner funnel, washed with 95 per cent alcohol, and dried at 35° . The yield was $8\frac{1}{2}$ g.

6. ACETYLATION OF β -MANNOSE- $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$

Ten grams of β -mannose- $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ was added in small portions during the course of one-half hour to a mixture of 50 ml acetic anhydride and 65 ml pyridine, previously cooled in an ice bath. The mixture was held in the ice bath with frequent shaking for four hours and then kept in an ice chest for two days. When it was poured on crushed ice, crystallization of β -mannose pentaacetate took place almost immediately. The crystals were filtered on a suction funnel, washed with water, and dried at 35° . The yield of this impure material was $5\frac{1}{2}$ g. The filtrate, after neutralizing with sodium bicarbonate and extracting with chloroform, gave a further $1\frac{1}{4}$ g. These two quantities of impure crystals were mixed and recrystallized from alcohol, yielding 6 g of pure β -mannose pentaacetate, or 50 per cent of the theoretical.

III. SUMMARY

Two crystalline isomeric double compounds of *d*-mannose with calcium chloride have been prepared.

One, designated the first isomer, shows an unusual course of mutarotation and an initial specific rotation which indicate that the sugar constituent of the double compound is a new form of mannose.

The extrapolated initial rotation of this compound shows a value for the mannose portion in good agreement with that previously calculated by Hudson for a β -mannose of 1, 5 ring structure.

A second isomer was obtained by evaporating an alcoholic solution of the first on a water bath. This product is a double compound of the previously known β -mannose and calcium chloride.

The preparation and study of compounds of the sugars with salts appears to offer a promising method for the discovery of new crystalline forms of the sugars.

WASHINGTON, February 13, 1929.