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RELATIONS BETWEEN ROTATORY POWER AND STRUCTURE IN THE SUGAR GROUP. XX. TWO ISOMERIC CRYSTALLINE COMPOUNDS OF *d*-MANNOSE WITH CALCIUM CHLORIDE[1]

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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 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 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

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[1] Published by permission of the Director of the Bureau of Standards, U. S. Department of Commerce. By arrangement with Dr. C. S. Hudson this article is included in his series entitled "Relations between Rotatory Power and Structure in the Sugar Group" as Number XX. Number XIX was published by Hudson and Isbell in THIS JOURNAL, 51, 2225 (1929).

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[3] v. Lippmann, "Chemie der Zuckerarten," 3d ed., pp. 549 and 884.

calcium and chlorine content and specific rotation at equilibrium corresponding to the formula $C_6H_{12}O_6 \cdot CaCl_2 \cdot 2H_2O$. Its initial specific rotation and course of mutarotation definitely correlate it with β-mannose of initial $[\alpha]_D -17°$; hence it will be designated as β-mannose$\cdot CaCl_2 \cdot 2H_2O$.

Initial Specific Rotation and Mutarotation of the First Isomer, Mannose$\cdot CaCl_2 \cdot 4H_2O$.—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 Levene[4] had found $[\alpha]_D +30°$ for α-mannose. Subsequent attempts to obtain readings more quickly after making the

TABLE I

MUTAROTATION OF THE FIRST ISOMER, *d*-MANNOSE$\cdot$CACL$_2\cdot$4H$_2$O, IN WATER

1.7437 g. of comp. in 25 cc. of soln.; tube length, 2 dm.; $T = 20 \pm 0.5°$

Time after making soln., minutes	$[\alpha]^{20}_{D}$ of compound	Time after making soln., minutes	$[\alpha]^{20}_{D}$ of compound		Time	$k_1 + k_2 = \frac{1}{T} \log \frac{r_0 - r_\infty}{r - r_\infty}$	
1.5	−6.22	8	+9.46	→	0	. . .	
3	+4.73	10	+9.08		2	0.032	
4	+7.21	12	+8.95		4	.022	
5	+8.74	14.5	+8.64		6.5	.024	
6	+9.20	17	+8.33		9	.026	Av. 0.025
7	+9.46	22	+7.80		12	.028	
		29	+7.71		21	.021	
		39	+7.09		31	.028	
		. .	+6.72				

TABLE II

OBSERVATIONS

2.856 g. of compound in 25 cc. of soln.; tube length, 2 dm.; $T = 2 \pm 0.5°$

Time after making soln., minutes	$[\alpha]^{20}_{D}$ of compound	Time after making soln., minutes	$[\alpha]^{20}_{D}$ of compound		Time	$k_1 + k_2 = \frac{1}{T} \log \frac{r_0 - r_\infty}{r - r_\infty}$	
0.75	−23.68	10.25	+ 6.68				
1.25	−17.60	12	+ 8.62				
1.5	−15.97	15	+10.50				
2	−12.75	18	+11.05				
2.25	−11.83	23	+11.35	→	0	. . .	
3	− 9.10	30.5	+10.93		7.5	0.0052	
3.5	− 7.89	40	+10.42		17	.0054	
4	− 5.46	50	+10.00		27	.0053	
4.5	− 4.55	63	+ 9.42		40	.0054	
5	− 2.73	79	+ 9.10		56	.0048	Av. 0.0052
5.5	− 1.82	93	+ 8.62		70	.0051	
6	+ 0.61	111	+ 7.71		88	[.0068]	
6.75	+ 1.82	130	+ 7.10		107	[.0085]	
7.5	+ 3.03	147	+ 6.68		124	[.0115]	
8.25	+ 4.85	∞	+ 6.50		∞	. . .	
9	+ 5.16						

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[4] Levene, *J. Biol. Chem.*, **57**, 329 (1923); **59**, 129 (1924).

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 measured accurately. At 2° it was still rapid but could be followed with sufficient precision to permit a close estimation of the initial value. In Tables I and II 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.

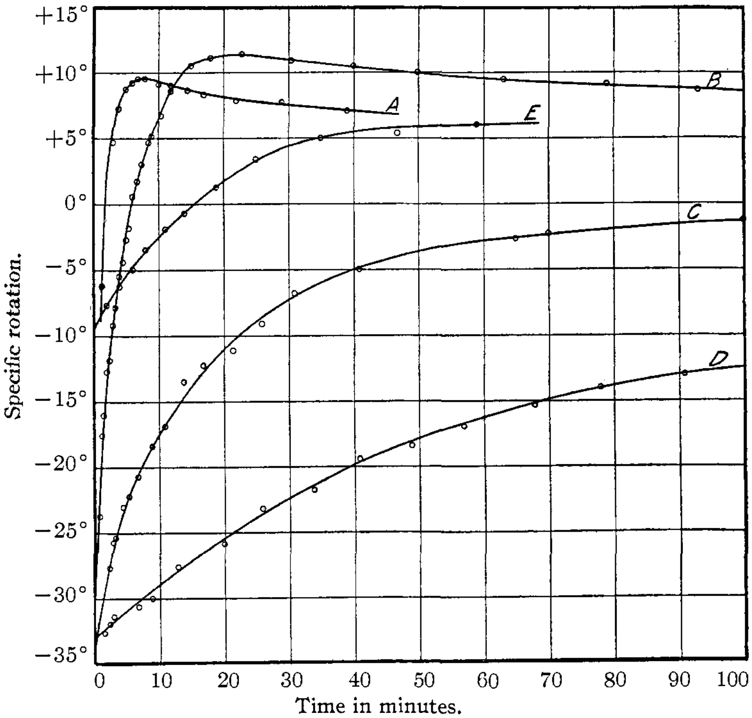

Fig. 1.—Initial stages in the mutarotation of mannose-calcium chloride compounds: Curve A, first isomer in water at 20°; Curve B, first isomer in water at 2°; Curve C, first isomer in methanol at 20°; Curve D, first isomer in methanol at 5°; Curve E, beta isomer in water at 20°.

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-mannose[5] (0.019), and Levene[4] has shown that α-mannose has the same rate of mutarotation as the β-form. Fur-

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[5] Hudson and Sawyer, This Journal, **39**, 475 (1917).

thermore, 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.

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.[6] *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. Hudson[7] 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 of $+30°$ 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 two-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 com-

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[6] 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.

[7] C. S. Hudson, THIS JOURNAL, **48**, 1425, 1434 (1926).

pound with calcium chloride 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 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.

Mutarotation of β-Mannose·$CaCl_2$·$2H_2O$.—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 III 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-mannose[5] at this temperature.[8] The final specific rotation, $+6.73°$, and

TABLE III

MUTAROTATION OF α-MANNOSE·$CaCl_2$·$2H_2O$ IN WATER

3.0146 g. of compound in 25 cc. of soln.; tube length, 2 dm.; $T = 20 \pm 0.5°$

Time after making soln., minutes	$[\alpha]^{20}_D$ of compound	Time	$k_1 + k_2$	
2	−7.70	0	. .	
4	−6.33	2	0.022	Av. 0.024
6	−5.02	4	.025	
8	−3.47	6	.025	
11	−1.97	9	.024	
14	−0.68	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	∞	. .	

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[8] The difference between the value $k_1 + k_2 = 0.024$ found for β-mannose·$CaCl_2$·$2H_2O$ 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·$CaCl_2$·$2H_2O$ 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 $k_1 + k_2 = 0.025$.

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°.

Acetylation of the $Mannose\cdot CaCl_2$ **Compounds.**—β-$Mannose\cdot CaCl_2\cdot 2H_2O$ may be of utility in the synthesis of mannose derivatives because it can be directly acetylated to give a good yield of β-mannose penta-acetate. This may be of timely importance, for pure β-mannose has recently become difficult to obtain, at least in some laboratories. Levene[4] 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 acetylated[9] to give alpha mannose penta-acetate, but the yield is not more than 20% of the theoretical, which is far from satisfactory. On the other hand, β-mannose or β-$mannose\cdot CaCl_2\cdot 2H_2O$ readily yields on acetylation 60% or more of the theoretical. The First Isomer ($mannose\cdot CaCl_2\cdot 4H_2O$) 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 penta-acetate.

Experimental

Preparation of the First Isomer, $Mannose\cdot CaCl_2\cdot 4H_2O$, **from** β-*d*-**Mannose.**—Twenty g. of β-mannose and 32 g. of crystallized calcium chloride dihydrate were dissolved on a boiling water-bath in 28 cc. of water. On standing overnight 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 cc. of cold water was filtered through an asbestos mat, followed by the addition of 10 cc. of water. The filtrate was placed in a crystallizing dish in a vacuum desiccator over sulfuric acid. 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% alcohol and in acetone. It melted at 101–102° corr.

Analysis.—Chlorine and calcium were determined directly in an aqueous solution of the compound. Moisture was determined by drying at 50 mm. over phosphorus pentoxide, first for six hours at 76°, then for nineteen hours at 100°. Four hours' additional drying at 110° caused no further loss in weight. Subs., 1.0197: AgCl, 0.8080. Subs., 1.0239: CaO, 0.1615. Calcd. for $mannose\cdot CaCl_2\cdot 4H_2O$: Cl, 19.56; Ca, 11.02; H_2O, 19.80; $[\alpha]_D^{20}$, 6.94°. Found: Cl, 19.62; Ca, 11.16; loss on drying to constant weight, 19.10; observed $[\alpha]_D^{20}$, + 6.72°.

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[9] Levene, *J. Biol. Chem.*, **57**, 329 (1923).

Preparation of the First Isomer, Mannose·$CaCl_2$·$4H_2O$, from Vegetable Ivory.—A mannose sirup was prepared essentially according to the methods of Hudson and Sawyer[5] and Clark[10] 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% sulfuric acid and kneaded into a thick paste. After standing at room temperature for twenty-four 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 3 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 sulfate was separated by filtration with suction and well washed. The mixed filtrate and washings were acidified with 2 cc. of 5% sulfuric acid 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% 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 cc. of water. The mixture was stirred to a uniform solution, which was then diluted with 135 cc. of equal parts of absolute alcohol and acetone. Crystallization began almost immediately after cooling and seeding. After one night at room temperature and twenty-four hours in an ice chest, the crystals were filtered off on a Büchner funnel and washed with 400 cc. 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·$CaCl_2$·$4H_2O$, as originally obtained from crystalline β-mannose.

Transformation of the First Isomer, Mannose·$CaCl_2$·$4H_2O$, into β-Mannose·$CaCl_2$·$2H_2O$.—Ten grams of the First Isomer was dissolved in 40 cc. 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. 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 overnight, filtered and the crystals washed with absolute alcohol. Dried at 35° there was obtained 6.5 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% alcohol and practically insoluble in absolute alcohol. The substance melted at 159–160° (corr.) with decomposition.

Analysis.—Chlorine and calcium were determined directly in aqueous solution. The results agree with the percentages calculated for mannose·$CaCl_2$·$2H_2O$, but holding at 50 mm. over phosphorus pentoxide at 100° for five hours caused no loss in weight. Subs., 0.9799: AgCl, 0.8550. Subs., 1.0168: CaO, 0.1741. Calcd. for mannose·$CaCl_2$·$2H_2O$: Cl, 21.71; Ca, 12.23; $[\alpha]^{20}_{\mathrm{D}}$, 7.7°. Found: Cl, 21.59; Ca, 12.23; obs. $[\alpha]^{20}_{\mathrm{D}}$, 6.73°.

Reversal of the above Transformation.—Ten grams of β-mannose·$CaCl_2$·$2H_2O$ was dissolved in 150 cc. of boiling absolute alcohol. The solution was cooled and 1.1 cc. of water was added, just 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 semi-solid 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.

Preparation of β-Mannose·$CaCl_2$·$2H_2O$ 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

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[10] Clark, *J. Biol. Chem.*, 51, 1 (1922).

from hydrolyzed vegetable ivory. In the former case 20 g. of β-mannose with 24 g. of calcium chloride dihydrate was dissolved in 12 cc. of hot water, the solution placed on a boiling water-bath, and 80 cc. 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% alcohol and dried at 35°, there was obtained 28 g.

The same procedure of crystallizing from strong, hot alcohol was applied to the preparation of this compound directly from an impure mannose sirup. Ten g. of a thick sirup from hydrolyzed vegetable ivory prepared as described earlier was mixed with 10 g. of $CaCl_2 \cdot 2H_2O$, previously dissolved in 3 cc. of water, on a boiling water-bath. Thirty-five cc. 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·$CaCl_2 \cdot 2H_2O$ 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 forty-eight hours at room temperature, the crystals were separated on a Büchner funnel, washed with 95% alcohol and dried at 35°. The yield was 8.5 g.

Acetylation of β-Mannose·$CaCl_2 \cdot 2H_2O$.—Ten g. of β-mannose·$CaCl_2 \cdot 2H_2O$ was added in small portions during the course of one-half hour to a mixture of 50 cc. of acetic anhydride and 65 cc. of 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 onto crushed ice, crystallization of β-mannose penta-acetate 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 5.5 g. The filtrate, after neutralizing with sodium bicarbonate and extracting with chloroform, gave a further 1.25 g. These two quantities of impure crystals were mixed and recrystallized from alcohol, yielding 6 g. of pure β-mannose penta-acetate, or 50% of the theoretical.

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 appear to offer a promising method for the discovery of new crystalline forms of the sugars.

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