The benzylphenyl hydrazones exhibit mutarotation and doubtless exist, therefore, in solution in isomeric forms and the rotations of their solutions refer to mixtures of such forms in equilibrium. Their structures are not as simple and as definitely known as are those of the acid phenylhydrazides, the rotations have not been measured in one solvent throughout, and the correlation between their structure and rotation is not proved in as many cases as have been shown for the phenylhydrazides. Nevertheless, the existing data indicate that such a relationship probably holds.

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[CONTRIBUTION FROM THE CARBOHYDRATE LABORATORY, BUREAU OF CHEMISTRY, UNITED STATES DEPARTMENT OF AGRICULTURE, AND THE PHYSICAL CHEMISTRY LABORATORY OF PRINCETON UNIVERSITY.]

THE PREPARATION OF PURE CRYSTALLINE MANNOSE AND A STUDY OF ITS MUTAROTATION.

BY C. S. HUDSON AND H. L. SAWYER.

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1. The Hydrolysis of Vegetable Ivory and the Direct Crystallization of Beta Mannose.*

Without doubt the best source from which mannose may be prepared is the waste sawdust and turnings from vegetable ivory button factories as has been pointed out by Reiss[1] and by Fischer and Hirschberger.[2] Vegetable ivory is the endosperm of the seed of the tagua palm, *phytelephas macrocarpa*, and by acid hydrolysis it yields large proportions of mannose. Hitherto, the usual method for preparing the sugar in crystalline condition has been the procedure of Fischer and Hirschberger, which consists essentially in hydrolyzing the vegetable ivory with 6% hydrochloric acid at 100° for six hours, neutralizing the solution with sodium hydroxide, decolorizing it with bone char and precipitating the mannose with phenylhydrazine as the insoluble mannose phenylhydrazone. The latter is purified by recrystallization and the mannose regenerated from it by boiling with benzaldehyde.[3] The sirup which is thus produced crystallizes slowly after being seeded with mannose crystals, which were first obtained by Van Ekenstein.[4] This method is expensive

* Our thanks are expressed to Mr. T. S. Harding for his skilful assistance in the development of this method for preparing crystalline mannose readily.

[1] *Ber.*, **22**, 609 (1889).

[2] *Ibid.*, **22**, 3218 (1889).

[3] Herzfeld and de Witt, *Z. Ver. Rübenzuckerind.*, **32**, 794 (1895).

[4] *Rec. trav. chim. Pays Bas.*, **15**, 222 (1896).

in making the sugar on a large laboratory scale on account of the intermediate preparation of mannose phenylhydrazone. It seemed possible to the authors that mannose might be crystallized directly from the hydrolyzed solution of vegetable ivory, and after experiments extending over several years, it has proved possible to accomplish this crystallization with a large yield.

We first sought to obtain as complete an hydrolysis as possible of vegetable ivory and although the following procedure was not the one which was finally adopted, its description may be of interest because the products of hydrolysis gave crystalline mannose directly and the process of hydrolysis is probably the least expensive that can be devised. Five hundred grams of finely ground vegetable ivory meal were added to a 2% solution of sulfuric acid, using one part of the meal to four parts of the acid solution. These materials were thoroughly mixed and heated at 145° to 152° in a copper autoclave under seventy-five to eighty pounds' pressure for twenty minutes with frequent shaking. The autoclave was then quickly cooled by immersion in cold water. The contents were removed and filtered, the filtrate neutralized with barium hydroxide, mercuric acetate added in slight excess as a clarifying agent and the combined precipitates, consisting of barium sulfate and a flocculent material, were filtered from the clear liquid. The excess of mercury was precipitated with hydrogen sulfide gas and the excess of the latter was removed by a current of air. In order to aid the decolorization by bone char at the following stage, it was found necessary to add sulfuric acid at this point to precipitate barium which is probably in combination with organic acids. The filtered liquid was then purified by passing it through bone char. The thick sirup obtained by concentrating the nearly colorless solution on a steam bath *in vacuo* slowly crystallized after seeding, and the crystallization was greatly hastened by adding an equal volume of glacial acetic acid to the sirup, following the excellent method which Wernicke and Pfitzinger[1] have used for the crystallization of sucrose. Several pounds of mannose were prepared in this way but the yield was small, only 10% of the weight of the vegetable ivory, and since the estimation of reducing sugar in the original hydrolysis showed that from 40% to 60% of the vegetable ivory had been converted to sugar, it seemed desirable to find other conditions of hydrolysis which might yield a larger proportion of crystallizable sugar.

The following procedure proved much more successful, and it is the one which we recommend for the preparation of crystalline mannose. It is a development of the method which Reiss first proposed, though he did not obtain the mannose crystalline. One hundred and fifty grams of sifted vegetable ivory meal are slowly added in small quantities, about 10 g.

[1] U. S. Patent No. 260,340 of June 27, 1882.

at a time, to 150 g. of a 75% solution of sulfuric acid at room temperature. After each addition the meal and acid are thoroughly mixed and the temperature is not allowed to rise above 40°. An irritating odor is noticeable during this treatment and the meal changes to a reddish brown and later to a purple color. The meal and acid are allowed to remain at room temperature with occasional stirring for at least three hours, preferably six, but not more than twenty, after which the mixture is diluted to two liters with water. Very little heat is evolved on the addition of water, indicating that the meal and acid have combined chemically. The diluted solution is transferred to a 2.5 liter flask connected with a reflux condenser and gently boiled three hours. Nearly all of the meal goes into solution during boiling. Several experiments were made to determine the quantity of meal in solution after hydrolysis, by weighing the flocculent residue. After washing and drying, it weighed 8 to 12 g., depending on the length of time the acid and meal had been in contact previous to dilution, and on the duration of boiling. For meal that had been treated with acid for 12 hours and diluted with water, then boiled for three hours, the weight of the precipitate remained fairly constant and indicated that the proportion of the vegetable ivory which is dissolved by this treatment is over 90% under good conditions. The rate at which reducing sugar is produced on boiling the solution corresponds to 60% of the meal in thirty minutes, 72% in one hour, and a constant value of 85% to 90% in two to three hours. A slow hydrolysis proceeds in the cold mixture of 75% sulfuric acid and meal, as was shown by determinations of reducing sugars in mixtures that had stood for varying lengths of time. It was found that meal which had been standing with 75% acid for twenty days gave on dilution as much as 32% of reducing sugar. When the material was allowed to stand only ten days, 20% to 25% of reducing sugar was found present. The usual period of reaction at room temperature was about six to twelve hours and on dilution analysis showed from 12% to 15% of reducing sugar.

Various attempts were made to purify the meal before it was mixed with 75% sulfuric acid. It was found that digesting it with a 1% solution of sodium or potassium hydroxide showed a marked action. Five hundred grams of meal were digested on the steam bath with a 1% solution of potassium hydroxide eight successive times or until the liquor was colorless, and it was found that only 30% of the meal remained. Meal thus cleansed did not change color appreciably or develop a pungent odor when treated with acid in the cold, but the yield of crystallized mannose was far below that obtained from the meal which had not been previously washed with dilute alkali. Hence, the method was abandoned, although it has some suggestive points bearing upon the composition of vegetable ivory.

After cooling the hydrolyzed solution, the next step consists in careful neutralization of the sulfuric acid with calcium hydroxide. It is important that the reaction should not be allowed to become alkaline. The calcium sulfate precipitate is filtered by suction through a Büchner funnel, and well washed with hot water. If the precipitation and washing have been carefully carried out, the loss of reducing sugar is only from ten to fifteen grams. The volume of the neutral solution at this stage is between two and three liters and the color a dark reddish brown, but on the addition of 0.5% of glacial acetic acid, the color changes to a light amber. The solution may now be decolorized with bone black, but it is preferable to accomplish this with the recently introduced vegetable decolorizing carbons, such as "eponit" or "norit." About 3 g. of the carbon are added to the solution, followed by a slight excess of basic lead subacetate solution (30 cc.), the precipitate is filtered off, the excess of lead is removed with H_2S gas and the filtrate is again treated with 3 g. of decolorizing carbon and filtered. The colorless solution is then evaporated under reduced pressure to a thin sirup, and 500 cc. of 95% alcohol are added after cooling. The precipitate of calcium salts is removed and the clear, nearly colorless, alcoholic solution is treated with H_2S, a black precipitate of iron sulfide is filtered off, and the filtrate is evaporated under reduced pressure to a sirup so thick that when the flask is removed from the steam bath and decanted the sirup moves slowly if at all. It should be brought as near to a solid mass as possible and yet be removable when an equal volume of glacial acetic acid is added and the flask warmed and shaken. After the sirup has cooled, a few crystals of mannose are added and with occasional stirring crystallization proceeds at room temperature and reaches completion in five to seven days. An equal volume of glacial acetic acid is added in small portions, 25 cc. at a time, as crystallization proceeds. The mannose is separated from its mother liquor in a Büchner funnel with suction, and the crystals are washed with glacial acetic acid. The sugar is quite white and the yield is from 35% to 40% of the weight of the vegetable ivory.

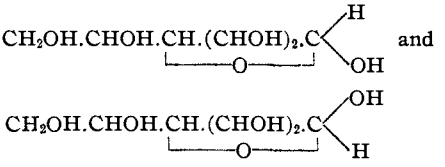
Mannose may be recrystallized either from glacial acetic acid or from 95% ethyl alcohol. To recrystallize from acetic acid, the crude sugar is dissolved to a thin solution in water and decolorized with carbon. The solution is now concentrated to a very thick sirup *in vacuo*, $1\frac{1}{2}$ to 2 volumes of glacial acetic acid added and crystallization accomplished as before. To recrystallize from ethyl alcohol, dissolve about 150 g. crude sugar in 500 cc. 95% alcohol on the steam bath, using a reflux condenser. Filter off any flocculent precipitate and concentrate the alcoholic solution to a moderately thick sirup on the steam bath. Upon seeding, crystallization takes place, is complete over night and gives a yield of 55–60%. The specific gravity at 20° of these crystals was found

1.539, and the initial rotatory power showed them to be the beta form of mannose.

The mannose thus obtained has initially a sweet taste followed immediately by a distinctly bitter one. This peculiar characteristic was the same for all of the numerous samples of purified mannose which we prepared and it agrees well with the observations of Van Ekenstein[1] and of Sternberg.[2] Neuberg and Mayer[3] maintain that when mannose is properly purified the bitter taste disappears. We were unable to verify their conclusion and feel confident that the mannose obtained by the new process herein described cannot be less pure than that which Neuberg and Mayer prepared. We conclude that the bitter after-taste of mannose is a characteristic of the pure substance.

2. Measurement of the Rate of Mutarotation of Mannose in Dilute Aqueous Solution at Temperatures from 0° to 45°.

It was found by Van Ekenstein[4] that crystalline mannose shows mutarotation, its specific rotation after dissolving in cold water being about $-14°$ and changing gradually during a few hours to become constant at $+14.25°$. From the fact that mannose is an aldose sugar it is to be expected that it should show mutarotation, since it probably exists, reasoning by analogy from the known alpha and beta forms of glucose, lactose, arabinose, galactose and rhamnose, in two modifications having the structural formulas



The existence of the isomeric crystalline beta[5] and alpha[6] pentacetates of mannose is further evidence that the sugar exists in two corresponding isomeric forms. Crystalline mannose is generally regarded as the beta form because it shows mutarotation towards the right-handed direction. The mutarotation of mannose may be considered as due to a balanced reaction

$$\text{alpha mannose} \rightleftarrows \text{beta mannose},$$

and the formulation of the rate of this reaction in the terms of chemical kinetics[7] gives the unimolecular order equation

[1] Van Ekenstein, *Rec. trav. chim. Pays Bas.*, **15**, 232 (1896).
[2] Sternberg, *Compt. rend.*, **138**, 191 (1904).
[3] Neuberg and Mayer, *Z. physiol. Chem.*, **37**, 547 (1903).
[4] *Loc. cit.*
[5] Fischer and Oetker, *Ber.*, **46**, 4029 (1913).
[6] Hudson and Dale, THIS JOURNAL, **37**, 1280 (1915).
[7] For the details, see *Ibid.*, **26**, 1067 (1904).

$$\frac{1}{t} \log \frac{r_0 - r_\infty}{r - r_\infty} = k_1 + k_2,$$

which is the general formula for the mutarotation of the various sugars. The rotation at the start is r_0, the final rotation is r_∞, the rotation at the time t after the start is r, and the constant $(k_1 + k_2)$ is the unimolecular coefficient. It has been shown by Pratolongo[1] that the course of the mutarotation of mannose follows this formula. We have measured the coefficient of the rate of mutarotation $(k_1 + k_2)$ at temperatures ranging from 0° to 45° and have obtained the values recorded in Table I. The mannose used was very pure, and had been crystallized either twice from glacial acetic acid, or once from the acid and once from alcohol, or several times from alcohol. The samples which had been purified in these different ways did not differ in their rate of mutarotation. The experiments were carried out in the usual manner. The mannose solutions were chosen of about 10% strength, since it was found in other experiments (see Table II) that the rate is independent of the sugar concentration at this and greater dilutions. The temperature is correct within 0.1°, the time is expressed in minutes and decimal logarithms are used.

TABLE I.—THE RATE OF MUTAROTATION OF MANNOSE IN DILUTE AQUEOUS SOLUTION AT TEMPERATURES FROM 0° TO 45°.

T.	Experiment I $(k_1 + k_2)$.	Experiment II $(k_1 + k_2)$.	Average $(k_1 + k_2)$.	Calculated value $(k_1 + k_2)$.
— 0.1	0.00241	0.00245	0.00243	[0.00243]
+ 1.0	...	...	0.0030[2]	0.0030
4.8	0.00400	0.00417	0.00408	0.00412
9.7	0.00656	0.00632	0.00644	0.00686
10.0	...	...	0.0066[2]	0.0076
14.7	0.0105	0.0113	0.0109	0.0113
19.7	0.0179	0.0175	0.0177	0.0184
20.0	...	...	0.019[2]	0.020
24.8	0.0289	0.0292	0.0297	0.0290
29.7	0.0464	0.0465	0.0464	[0.0464]
30.0	...	...	0.049[2]	0.049
34.8	0.0676	0.0688	0.0682	0.0724
39.7	0.1053	0.1066	0.1062	0.1108
44.8	0.161	0.182	0.171	0.167

Pratolongo[3] found the velocity coefficient in 15% mannose solution to have the values 0.0226 at 21.1° and 0.0245 at 21.6°, which agree with our data within the limits of measurement.

[1] *Rend. Inst. Lombardo*, **45**, 975 (1912).

[2] These results were obtained in 1910 by one of us (Hudson). The mannose used in these earlier experiments was prepared by crystallization from aqueous solution, followed by recrystallization from aqueous alcohol. The measurements are in close agreement with the results which were obtained in 1913 by the other author (Sawyer).

[3] *Loc. cit.*

The calculated results in the last column of the table are derived from the integrated formula of van't Hoff, $\log (k_1 + k_2) = C - A/T$ where C and A are constants and T is the absolute temperature. Using the rates of mutarotation at —0.1° and at 29.7°, to determine the constants A and C, the formula becomes $\log_{10} (k_1 + k_2) = 10.3903 - 3549/T$. The average increase in the velocity coefficient for a ten-degree rise in temperature is 2.6 fold.

3. The Variation of the Rate of Mutarotation with the Concentration of Sugar.

Experiments were performed to determine the relation between the concentration of the mannose solution and the corresponding velocity coefficient. A quantity of sugar was dissolved quickly in distilled water at a temperature of 19.7° and the solution filtered, poured into the polariscope tube and readings taken at short intervals. The temperature was kept 19.7° during the mutarotation. The following table shows the change in the velocity coefficient with increasing concentration. In dilute solution the rate is independent of the concentration but a slight increase of the rate is noticeable at or about twenty grams sugar. The rate increases steadily with the concentration until a maximum is reached at forty-five grams sugar and in higher strengths the rate rapidly decreases. Higher concentrations than fifty-six could not well be obtained at this temperature, 19.7°. The influence of the concentration on the rate is quite similar to that which was recently observed in the case of glucose.[1]

TABLE II.—THE CHANGE IN THE RATE OF MUTAROTATION WITH VARYING CONCENTRATIONS OF MANNOSE.

Grams of mannose per 100 cc. solution.	$k_1 + k_2$.	Grams of mannose per 100 cc. solution.	$k_1 + k_2$.
5.13	0.0177	27.1	0.0192
8.0	0.0179	36.8	0.0197
10.0	0.0175	45.0	0.0200
10.2	0.0178	50.0	0.0192
19.1	0.0181	52.0	0.0189
24.7	0.0190	56.0	0.0179

There was noticed in these measurements a peculiar relation of the final to the extrapolated initial rotation. In more than thirty measurements which had been carried out in water, dilute alcoholic or acidic solution, with less than fifteen per cent. of mannose concentration, the ratio of the final to the initial rotation had a constant value of 1:—1.1. An increase in the concentration of the sugar altered this ratio as the following table shows:

[1] Hudson and Dale, THIS JOURNAL, 39, 325 (1917).

TABLE III.

Grams mannose per 100 cc. solution.	Extrapolated initial rotation.	Final rotation.	Ratio.
5	— 8.5	+ 8.3	1 : —1.0
20	—17.0	+15.5	1 : —1.1
24.7	—25.5	+20.0	1 : —1.2
30	—26.1	+21.9	1 : —1.2
50	—45.4	+20.2	1 : —2.2
52	—39.8	+21.0	1 : —1.9
56	—88.9	+45.1	1 : —1.9

To account for this marked change, it may be assumed that the specific rotation of alpha or of beta mannose or the equilibrium of their mutarotation reaction changes greatly with the sugar strength. A change in the equilibrium appears the more probable cause.

4. The Catalytic Action of Hydrochloric Acid on the Rate of Mutarotation.

It is well known that the rate of mutarotation of the sugars is greatly accelerated by the addition of hydrochloric or other acids. It is also true that alkalies hasten this reaction to a more marked degree than acids. We find that the mutarotation of mannose is catalyzed in similar manner by acids and by alkalies, a drop of ammonium hydroxide bringing about an equilibrium value almost instantly in solutions of the sugar. While the rate of change was not measured in the presence of alkalies on account of its extreme rapidity, several experiments were made in dilute solutions of hydrochloric acid. Pure crystalline mannose was dissolved quickly in the acid solution of appropriate strength, the solution filtered and the angle of rotation observed at short intervals of time. The mutarotation coefficient was calculated as previously described.

TABLE IV.—RELATION BETWEEN THE ACID CONCENTRATION AND THE RATE OF MUTAROTATION.

Temperature 19.7°.

Acid (HCl) concentration.	$k_1 + k_2$.	$\frac{\text{Rate increment}}{\text{Acid increment}}$
Distilled Water	0.0177	..
0.001 normal	0.0190	1.3
0.010	0.0396	2.19
0.0125	0.0460	2.21
0.0166	0.0558	2.29
0.025	0.0708	2.12
0.05	0.125	2.16
0.10	0.238	2.21

The first value in Col. 3 is somewhat lower than for the higher acid concentrations. The proportional increase is not as great, owing doubtless to the fact that acids in very dilute solutions may retard the rate of mutarotation, or not increase it in proportion to the acidity, as has been

shown for solutions of glucose.[1] For acid solutions of greater strength than 0.001 N the rate of mutarotation is a linear function of the acidity, which is shown by the fact that the ratio in Col. 3 is a constant.

5. Summary.

A method has been devised for crystallizing mannose directly and in large yield from the products of acid hydrolysis of vegetable ivory, without the use of phenyl hydrazine. Pure mannose has a slightly sweet taste followed by a distinctly bitter one. The specific gravity of the beta form of mannose is 1.539 at 20°. The rate of mutarotation of mannose in aqueous solution has been measured at temperatures from 0° to 45°, found to follow the unimolecular order, as shown previously by Pratolongo, and to increase 2.6 fold in speed for a rise of ten degrees in temperature. The rate of mutarotation in aqueous solution is independent of the sugar concentration below about ten per cent. sugar, but with higher sugar strengths the rate increases, reaches a maximum, and then decreases. Hydrochloric acid catalyzes the rate of mutarotation and the increase in rate is proportional to the increase in acidity within the range of tenth to thousandth normal. Ammonia has a far stronger catalytic action, as has been found for other sugars. Our results indicate that the mutarotation of mannose is a similar reaction to the mutarotation of the other aldose and ketose sugars and is caused by a balanced reaction alpha mannose $\rightleftarrows$ beta mannose.

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EVIDENCE OF THE ACTION OF OXIDASES WITHIN THE GROWING PLANT.

BY JOSEPH H. KASTLE[2] AND G. DAVIS BUCKNER.

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In reviewing the literature on the subject of oxidases we were unable to find any experimental evidence of oxidation occurring within the cells of growing plants. The phenomenon of root oxidation has been demonstrated experimentally, as has also the existence of oxidases in the expressed sap of different plants and in the dead plants themselves.

The following extract from Pfeffer credits the above:

"The reactions given by dead cells, or by the expressed sap, form no sure indication as to the conditions existing in the living cell, for in the latter, substances may be kept apart which react when in contact, as, for example, when a glucoside and a glucoside enzyme are present in the same cell. Various post-mortem oxidations may occur after death, as, for example, when the sap of *Monotropa, Vicia faba,* etc., turns brown.

[1] Hudson, THIS JOURNAL, 29, 1573 (1907).

[2] These experiments were made during the summer of 1914 but were not prepared for publication until after the death of Dr. Kastle.