THE REACTIVITY OF THE METHYLATED SUGARS. VI. THE ACTION OF DILUTE ALKALI ON 3-METHYLGLUCOSE[1]

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It has been previously shown[2] that when tetramethylglucose is treated with lime water, tetramethylmannose is formed but no methylated ketose. Moreover, the reaction mixture shows a higher iodine absorption than can be accounted for by the presence of the aldoses. When the reaction mixture is treated with acid, the iodine absorption drops to a value corresponding to 100% aldose. These results were explained by postulating that because of the methyl group on the second carbon atom in tetramethylglucose, only the 1,2-enediol can form, in this case a monomethyl-enediol, thus limiting the alkali conversion to the epimeric tetramethylmannose and precluding the formation of ketose. The high iodine absorption value of the neutral or alkaline reaction mixture is believed to be due to the presence of an enol stabilized by methyl groups even as methylation stabilizes the parent sugar. Acidification, bringing the iodine absorption down to 100%, reflects a de-enolization.

Similarly, tetramethylmannose[3] was shown to yield only tetramethylglucose under the same conditions, with the same evidence of enolization and de-enolization. In both cases the corresponding unmethylated sugars, glucose and mannose, respectively, were shown to produce fructose with some saccharinic acid, but no stable intermediate enol under the same conditions.

If the foregoing considerations are correct, it follows that if the hydroxyl on the second carbon in a methyl glucose remains unmethylated, the 1,2-enediol resulting from alkali treatment would also be unmethylated and therefore capable of forming a ketose according to the accepted explanation of the shifting hydrogen atom of an enol. Furthermore, if only the hydroxyl on the third carbon atom in such a methylated glucose were methylated, the 2,3-enediol could form, in addition to the 1,2-enediol, but further enolization would be blocked. The 2,3-enol so formed would be a monomethyl-enediol and therefore its stability under the conditions as evidenced by iodine absorption would become a point of interest. If the methylated ketose thus formed should lend itself to isolation, the
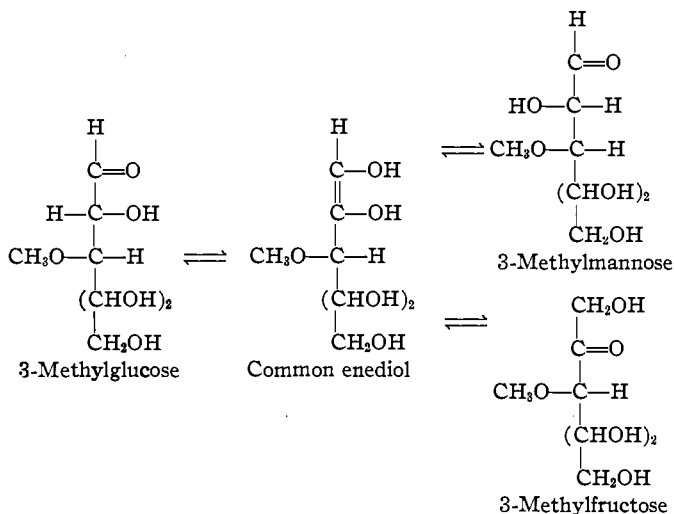
[2] Wolfrom with Lewis, THIS JOURNAL, 50, 837 (1928).

[3] Greene with Lewis, ibid., 50, 2813 (1928).

method suggests a source of new methylated ketoses and aldoses not now known, by dilute alkali conversion of the complementary methylated aldoses and ketoses.

3-Methylglucose offered the desired structure.   The 3-methylfructose[4] to be expected is a well-defined compound.   With the exception of the desired methyl group on the third carbon atom, 3-methylglucose most nearly resembles the parent glucose of any possible methylated sugar. Sobotka[5] has already given evidence from oxidation that the single methyl group blocks complete enolization of the sugar.   The following system is therefore to be expected when 3-methylglucose is treated with dilute alkali

$$
\begin{array}{ccccc}
 & & & & \text{3-Methylmannose} \\
\text{3-Methylglucose} & & \text{Common enediol} & & \text{3-Methylfructose}
\end{array}
$$

The above enediol group being unmethylated like that from glucose, should not yield a high iodine absorption on equilibration of the mono-methylglucose with alkali.   Should enolization proceed down the carbon chain to form the possible 2,3-mono-methyl-enediol (A), the high iodine absorption of a stable methylated enol would be expected.   Therefore, a normal iodine consumption of the equilibrated solution before acidification would give evidence that the 2,3-monomethyl-enediol did not form.
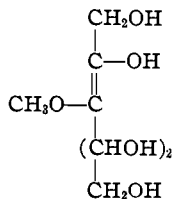
When 3-methylglucose was treated with lime water under the conditions of conversion used throughout these studies it was found to behave very much like glucose itself save that equilibrium was reached in forty-seven hours whereas in the case of glucose the changes are not complete at the end

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[4] Ohle and Koller, *Ber.*, **57**, 1571 (1924).

[5] Sobotka, *J. Biol. Chem.*, **69**, 267 (1928).

of one hundred sixty-three hours. Abnormal iodine absorption was not found from which it is concluded that lime water is not a sufficiently strong alkali to cause enolization beyond the carbon atoms 1 and 2. That is, the stable 2,3-monomethyl-ene-diol was not present. Some methanol was split off, probably incident to a small amount of saccharinic acid formation. Excess of lime and higher temperatures, or stronger alkalies at a temperature of 35°, caused rapid and extensive changes in 3-methylglucose with considerable loss of methanol. With lime water at 35° the drop in P_H and the loss of methoxyl corresponded roughly to the saccharinic acid formed, namely, 3.5% of the original sugar as calculated from the titration of the reaction mixture after removal of calcium ions. The aldohexose content as determined by iodimetry was 64.1%. This, with the 3.5% of saccharinic acid, would indicate that 32.4% of the original sugar must have been transformed into ketohexoses. $[\alpha]_D^{20}$ of the mixture calculated on the assumption that only 3-methylglucose and 3-methylfructose were formed was +15.9°. The observed value was 15.6°. The fact that the calculated value checks closely with that observed, when it is further known that saccharinic acid formation took place to the extent of 3.5% of the original sugar, suggests the presence of a small amount of a low rotary sugar, probably 3-methylmannose. This was not isolated. In any event the iodimetric value and the specific rotation of the mixture indicate that the products of alkali conversion are mainly 3-methylglucose and 3-methyl-fructose. It is noteworthy that glucose yields glucose and fructose in the same proportion under the same conditions.[2] The resemblance between the behavior of 3-methylglucose and glucose as well as the greater speed of conversion of 3-methylglucose are shown in Fig. 1.

TABLE I

RATE OF TRANSFORMATION OF 3-METHYLGLUCOSE INTO 3-METHYLFRUCTOSE

Time in hours	$[\alpha]_D^{20}$	Per cent. of glucose changed to fructose
..	55	0
12	25.5	25.2
17	23.2	28.4
34	17.3	35
47	15.9	35.1
288	15.6	32.4[a]

[a] Final value corrected for saccharinic acids.

From the reaction mixture 3-methylfructose was isolated in pure crystalline form. This establishes that the methyl group in monomethyl-fructose obtained by the methylation and hydrolysis of diacetone-fructose is located in the same position as in monomethylglucose. This latter has been proved to be on the third carbon atom but the position of the methyl in monomethylfructose has rested on the likeness of the osazones of the

two monomethyl sugars.   However, the variation in melting points of 3-methylglucosazone[6] has lent uncertainty to the proof.

3-Methylglucose was separated from the reaction mixture as the calcium salt of 3-methylgluconic acid and identified as the brucine salt.   A small fraction of brucine salts showed very different properties from those of 3-methylgluconate, indicating the presence of small amounts of other acids, probably saccharinic and 3-methylmannonic.
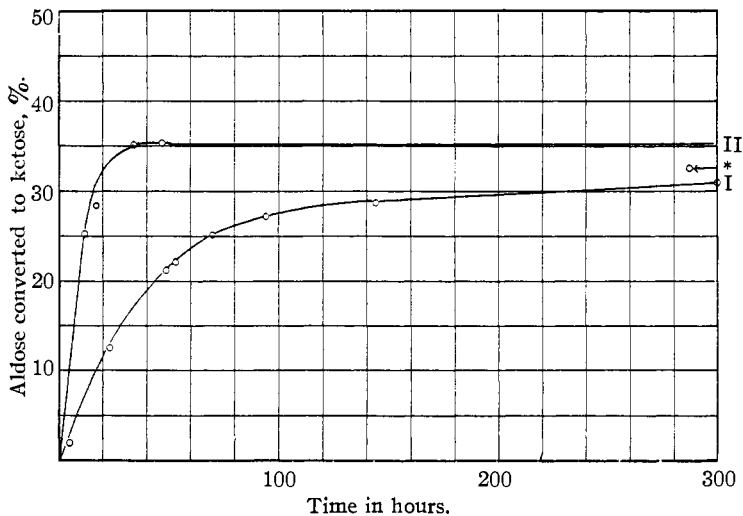

Fig. 1.—Comparison of the rates of transformation of glucose (I) and 3-methylglucose (II).   * Final value on 3-methylglucose corrected for saccharinic acids.

Experimental Part

Preparation of 3-Methylglucose.   Diacetone-glucose.—The method finally employed in the preparation of diacetone-glucose was a combination of various methods, principally those of P. A. Levene,[7] Karl Freudenberg[8] and H. Ohle.[4]   To 10 liters of pure acetone contained in a 12-liter flask was added with rapid mechanical stirring 100 cc. of concd. sulfuric acid.   Five hundred grams of crystalline glucose[9] was then added and the mixture stirred very rapidly with moisture excluded for about forty-eight hours.   Without removing the undissolved sugar, the acid was neutralized by passing a stream of dry ammonia gas into the mixture until the solution was faintly alkaline to litmus, meanwhile stirring rapidly and cooling under running water.   The

[6] (a) Irvine and Hynd, J. Chem. Soc., 95, 1220 (1909);   (b) Irvine and Scott, ibid., 103, 573 (1913);   (c) Anderson, Charlton, Haworth and Nicholson, ibid., 132, 1337 (1929);   (d) Freudenberg and Hixon, Ber., 56, 2126 (1923).

[7] Levene and Meyer, J. Biol. Chem., 57, 317 (1923);   Levene and Raymond, ibid., 83, 619 (1929).

[8] Freudenberg and Ivers, Ber., 55, 933 (1922).

[9] The product used is prepared on a commercial scale by the Corn Products Refining Co. and sold under the trade name "cerelose."

precipitate was then filtered with suction and washed with a little acetone. The acetone was evaporated rapidly from the solution under diminished pressure and the residue finally heated on the boiling water-bath for half an hour. The residue, which usually became solid, was repeatedly extracted with boiling ligroin (b. p. 80–90°). The extracts were then allowed to cool in the refrigerator and the crystalline mass filtered and washed with a little ligroin. The weight of the crude, colorless, diacetone glucose was generally about half the weight of the sugar taken. The crude material was dissolved in the minimum quantity of cold benzene and the resulting solution washed twice with 6 N sodium hydroxide solution and twice with water. The benzene was then evaporated under diminished pressure, the temperature being so regulated as to avoid crystallization, the last of the benzene being removed on a boiling water-bath under diminished pressure. In case crystallization had taken place, the residue was heated until complete solution resulted and about ten volumes of boiling ligroin (b. p. 80–90°) added. The solution, while still hot, was nucleated with a crystal of diacetone-glucose and the mixture shaken gently during the cooling, crystallization being completed in the refrigerator. The crystalline mass was filtered with the aid of suction, washed with low boiling petroleum ether and air dried. The product prepared in this way was pure white and melted correctly at 109–111°; reported, 110–111°.[10]

Diacetone-3-methylglucose.—The method developed by Freudenberg[11] for methylating diacetone sugars was used with only slight modification. In a 3-liter distilling flask were placed 569 g. (2.15 moles) of pure diacetone-glucose and 2.5 liters of dry benzene. The solution was then added with cooling to 70 g. of finely divided sodium in a 3-liter flask and the mixture allowed to stand overnight under a reflux condenser, care being taken to exclude air. The next morning the mixture was heated under a reflux condenser for about an hour and allowed to stand for three hours with frequent shaking. The solution was then transferred to a dry 3-liter distilling flask with the aid of suction, great care being taken to exclude moisture. The sodium residue was washed with dry benzene and the washings added to the main solution. Most of the benzene was then removed by distillation under diminished pressure at 50°. To the cooled, viscous solution was then added 600 g. of methyl iodide, and the mixture shaken thoroughly and cooled from time to time. On standing for two days the whole mass solidified. The precipitated sodium iodide was dissolved by adding 600 cc. of water and some benzene to the reaction flask. The benzene layer was separated, washed once with water, and dried with anhydrous potassium carbonate. The remaining benzene was removed by distillation from a one-liter Claisen flask and the crude diacetone-glucose fractionated. The product boiled at 103–106° with a bath temperature around 123° and a pressure of 4 mm. To the distilled diacetone derivative were added 28 g. of methyl iodide and 23 g. of silver oxide. The mixture was then warmed on the water-bath overnight. The product was separated from the silver oxide and redistilled: yield, 500 g., or 84% of the theoretical amount.

3-Methylglucose. Hydrolysis of Diacetone-3-methylglucose.—In a 3-liter flask was placed 1600 cc. of an equal mixture of methyl alcohol and water containing 17.6 g. of concentrated sulfuric acid. To this was added 100 g. of pure diacetone-3-methylglucose and the mixture heated on the water-bath for three hours. The acid was then neutralized with barium carbonate and the filtered solution evaporated under diminished pressure to a thick sirup which, after the addition of an equal volume of methyl alcohol, was nucleated with a crystal of 3-methylglucose. When the crystallization was practically complete, a little acetone was added and the mixture left in the refrigerator overnight. The crystals were filtered with the aid of suction, washed with a little

[10] E. Fischer and C. Rund, Ber., 49, 93 (1916).
[11] Freudenberg and Hixon, ibid., 56, 2119 (1923).

methyl alcohol, and dried *in vacuo* over sulfuric acid: yield, 54 g., or 76% of the theoretical amount; $[\alpha]_D^{25}$ +55.2° in sixteen hours (α = +4.48°, 2 dcm., c = 4.06 in water). The 3-methylglucose used finally in the equilibration experiment was carefully dried at 90° *in vacuo* over phosphorus pentoxide.

Anal. Calcd. for $C_7H_{14}O_6$: OCH_3, 15.95. Found: OCH_3, 15.90.

$[\alpha]_D^{20}$ +74.0°, initial; $[\alpha]_D^{20}$ + 55.0°, final (α = +4.70°, 2 dcm., c = 4.27 in water); recorded,[12] $[\alpha]_D^{17}$ + 104.3°, initial, and 55.3° after 19.5 hours.

Preparation of 3-Methylfructose. **α-Diacetone-fructose.[4]**—A mixture of 225 g. (1.25 moles) of fructose (Pfanstiehl 90%) and 4500 cc. of acetone containing 10 cc. of concentrated sulfuric acid was stirred rapidly for about thirty-six hours, when complete solution had usually taken place. The acetone solution was then made slightly alkaline by passing a stream of dry ammonia gas into it with rapid stirring. The filtered solution was evaporated under diminished pressure at 40° to dryness. The residue was extracted with 1 liter of ether, the ether extract washed with 300 cc. of 6 N sodium hydroxide solution and dried with 100 g. of anhydrous potassium carbonate. The ether solution was then concentrated under diminished pressure to a volume of about 200 cc., cooled in the refrigerator, the resulting crystalline mass filtered with the aid of suction and washed with a little ether. It was then heated to boiling with 500 cc. of petroleum ether (b. p. 63–93°). After the mixture had stood in the refrigerator overnight the product was filtered, washed and dried: yield, 102 g., or approximately 30% of the theoretical amount; $[\alpha]_D^{25}$ −159° (α = −4.36°, 1 dcm., c = 2.74 in water), reported, $[\alpha]_D$ −162.8°.[13]

Preparation of α-Diacetone-3-methylfructose.—The method used in methylating the diacetone-fructose is that of Freudenberg and Hixon[11] with slight modifications. To 500 cc. of dry benzene in a 1-liter distilling flask was added 25 g. of finely divided sodium metal. To this mixture was added in three portions 100 g. of diacetone fructose. It was necessary to cool the flask somewhat. The next morning the benzene was removed by distillation under diminished pressure at 35–40° and to the sirupy residue was added with shaking 110 g. of methyl iodide. The mixture stood for forty-eight hours, when 500 cc. of absolute ether was added and the solution decanted from the sodium into a 1-liter flask. The ether solution was filtered with considerable difficulty and the sodium iodide residue washed with ether and finally extracted with 200 cc. of benzene. (In later experiments the difficulty of filtering was avoided by decanting the benzene solution into a dry distilling flask with the aid of suction, thus separating the sodium. Water was then added to the ether solution to dissolve the sodium iodide. Ordinary ether may be used here.) The ether was removed by distillation, using diminished pressure finally. During the distillation the compound crystallized. After standing in the refrigerator overnight the crystals were filtered, washed with a little ether and dried; weight, 87 g. The filtrate was evaporated with the benzene extract under diminished pressure, taken up in a little absolute ether and placed in the refrigerator; weight, 10 g.; total yield, 97 g., or 92% of the theoretical; m. p., 114–115°; reported, 114–115°—(Ref. 11, p. 1343).

Preparation of 3-Methylfructose. Hydrolysis of α-Diacetone-3-methylfructose.— To 1200 cc. of an equal mixture of water and methyl alcohol containing 13 g. of concentrated sulfuric acid was added 60 g. of α-diacetone-3-methylfructose and the mixture heated for two hours on the steam-bath. The acid was neutralized with barium carbonate and the filtered solution evaporated under diminished pressure to a sirup.

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[12] Anderson, Charlton and Haworth, *J. Chem. Soc.*, **132**, 1329 (1929).

[13] Fischer, *Ber.*, **28**, 1164 (1895); Irvine and Hynd, *J. Chem. Soc.*, **95**, 1223 (1909); Irvine and Patterson, *ibid.*, **121**, 2160 (1922).

The sirup was concentrated twice with 200 cc. of methyl alcohol. To the sirup was added 200 cc. of acetone, part of which was then removed by distillation. Crystallization was completed by the addition of a little dry ether. The product was filtered, washed and dried in the usual way: yield, 35 g., or 82% of the theoretical amount. $[\alpha]_D^{20}$ $-54.0°$ in twelve hours ($\alpha = -4.52°$, 2 dcm., $c = 4.18$ in water); reported $[\alpha]_D^{20}$ $-53.5°$ ($c = 0.92$).[13]

Preparation of the Alkaloidal Salts of 3-Methylgluconic Acid.—In a 1-liter florence flask 19.4 g. of 3-methylglucose was dissolved in 750 cc. of water and oxidized according to the method of Hudson and Isbell.[14] The oxidized solution was finally extracted six times with 100-cc. portions of chloroform, made up to a volume of 1000 cc. and split into two equal portions, one to be used in preparing the cinchonine salt and the other the brucine salt of 3-methylgluconic acid. One-half of the oxidation solution was evaporated in a tared 500-cc. flask to a gum which was dried by heating under diminished pressure at 110° for two hours. The weight of the gum was 9.5 g.; theoretical weight, 9.6 g. $[\alpha]_D^{25}$ $+38.4°$ ($\alpha = +0.78°$, 1 dcm., $c = 2.03$ in water). The equivalent weight was 206, the calculated equivalent weight of the lactone being 192 and of the acid 210. The lactone could not be crystallized and did not give a crystalline phenylhydrazide.

The gum (9.16 g.) was dissolved in 200 cc. of water, 18 g. of recrystallized brucine added and the mixture heated on the water-bath until complete solution took place. The water was then evaporated under diminished pressure until a thick sirup was obtained to which 200 cc. of absolute ethyl alcohol was added and the mixture placed in the refrigerator. The crystals were filtered with the aid of suction, washed with absolute alcohol and air dried; yield, 21.7 g., or 78% of the theoretical amount. The product was recrystallized from a 50% water–alcohol mixture and dried over phosphorus pentoxide *in vacuo* at 98°; m. p. 146–148°, heated from 135° at 3° per min.; $[\alpha]_D^{25}$ $-16.7°$ ($\alpha = -1.68°$, 2.2 dcm., $c = 4.56$ in water).

Anal. Calcd. for $C_{23}H_{26}N_2O_4 \cdot C_7H_{14}O_7$: N, 4.63. Found: N, 4.64.

The second half of the oxidized glucose solution was heated on the steam-bath with 18 g. of pure cinchonine for ten hours with rapid mechanical stirring, at the end of which time the solution had taken up 11.9 g. of cinchonine of the theoretical 14.7 g. The filtered solution was evaporated under diminished pressure to a sirup which was dissolved in 300 cc. of absolute alcohol and the small precipitate removed by filtration. The filtrate was evaporated again to a sirup which was taken up in a small amount of absolute alcohol and allowed to crystallize in the refrigerator. The product was separated by filtration, washed with acetone and dried; yield, 17.5 g., or 70% of the theoretical amount. The salt was recrystallized once from absolute alcohol. The recrystallized product was not completely soluble in water, indicating that the absolute alcohol had reacted to some extent with the salt. The insoluble substance was separated by dissolving the salt in 30 cc. of water and filtering. The filtrate was evaporated under diminished pressure to a small volume and placed in the refrigerator to crystallize. The crystals were filtered and dried, first in the air and then at 76° *in vacuo* over phosphorus pentoxide. The product, although crystallizing readily, did not possess a definite melting point, but decomposed with evolution of gas at temperatures depending on the rate of heating; $[\alpha]_D^{25}$ $+113°$ ($\alpha = +6.24°$, 1 dcm., $c = 5.51$ in water).

Preliminary Study of Methods of Identification and Separation.—It was found that Cajori's[15] method for determining aldoses in the presence of ketoses by iodimetry was

[14] Hudson and Isbell, THIS JOURNAL, **51**, 2225 (1929).

[15] Cajori, *J. Biol. Chem.*, **54**, 617 (1922).

applicable to mixtures of 3-methylglucose and 3-methylfructose. 3-Methylglucose is rapidly oxidized by excess 0.1 N iodine in the presence of sodium carbonate while 3-methylfructose is but slowly attacked by the reagent under the same conditions. The rates of oxidation are about equally affected by change in temperature. At 25°, under the conditions employed, 3-methylglucose was 95% oxidized in thirty minutes. The rate of oxidation from that point was very slow. 3-Methylfructose was attacked more rapidly when alone, than in the presence of 3-methylglucose. At the end of thirty minutes, iodine corresponding to 1.6% aldose was used. A thirty-minute period at 25° was adopted. To apply the method to mixtures of 3-methylglucose and 3-methylfructose of different concentrations, it was necessary to establish an empirical relation between the iodine consumed and the relative quantities of the two sugars present. To do this it was only necessary to employ samples of mixtures of the two sugars under the same conditions and using the same total sugar concentration. The percentage of aldose shown by the amount of iodine used was then plotted with the actual concentration of the two sugars. The experiment was carried out in the following way. Solutions of 3-methylglucose and 3-methylfructose, each containing 5 mg. per cc., were prepared and mixed accurately in varying proportions, the total volume always being 20 cc. and containing a total of 0.1 g. of the two sugars. To the 20 cc. of sugar solution contained in a 500-cc. glass-stoppered flask was added 25.00 cc. of 0.09135 N iodine solution and 6 cc. of 15% sodium carbonate solution. The mixture was shaken and placed in the dark for exactly thirty minutes at 25°. The carbonate was then neutralized by adding a slight excess of 10% sulfuric acid. The excess iodine was then titrated with 0.05591 N sodium thiosulfate solution. The data obtained are shown in Table II.

TABLE II

OXIDATION OF MIXTURES OF 3-METHYLGLUCOSE AND 3-METHYLFRUCTOSE

Thiosulfate, 0.05591 N Iodine, 0.09135 N

3-Methyl-glucose soln., cc.	3-Methyl-fructose soln., cc.	Blank	Thiosulfate, cc.	Difference	3-Methyl-glucose from I₂, mg.	3-Methyl-glucose present, mg.	3-Methyl-fructose present, mg.
20	0	40.90	23.25	17.65	95.7	100	0
18	2	40.90	24.90	16.00	86.8	90	10
16	4	40.90	26.60	14.30	77.6	80	20
15	5	40.90	27.50	13.40	72.8	75	25
14	6	40.90	28.20	12.70	68.9	70	30
12	8	40.90	29.90	11.00	59.6	60	40
					1.6	0	100

These data are plotted in Fig. 2 and may be used for converting observed aldose content into actual aldose content when the conditions employed in preparing the chart are strictly adhered to in the determination. The application of the method to the equilibrated solution necessarily depends upon the assumption that only 3-methylglucose and 3-methylfructose are formed by the action of lime water, an assumption which is not strictly correct.

After a preliminary study of the separation of glucose and fructose by means of oxidation, formation of the calcium salt and solution of the fructose in alcohol, the method was applied to known mixtures of 3-methylglucose and 3-methylfructose. The residual sirup from the evaporated alcohol extracts easily yielded crystalline 3-methylfructose on inoculation but the lime salts of 3-methylgluconic acid were invariably high in calcium. Finally, by making the solution of 3-methylgluconic acid just alkaline to phenolphthalein with lime water, after neutralizing with calcium carbonate and

then discharging the color with oxalic acid and filtering, it was possible to get a lime salt of correct calcium content.   This salt was thrown out of the concentrated neutralized oxidation mixture by the addition of twenty volumes of methyl alcohol followed by a like amount of acetone with vigorous stirring.   A second precipitation was usually necessary to remove final traces of reducing monomethylfructose.
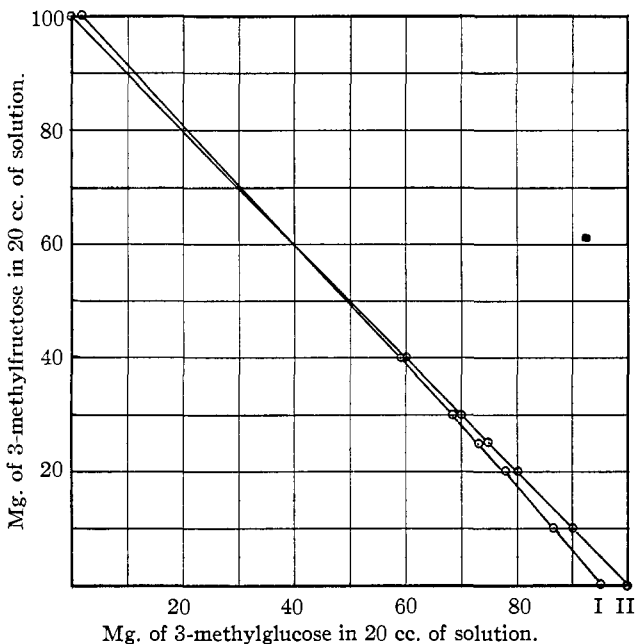

Fig. 2.—Reference curves for determining concentration of 3-methylglucose in the presence of 3-methylfructose: I, observed concentration; II, actual concentration.   Concentration of total sugar, 5 mg. per cc.

The Equilibration of 3-Methylglucose with Lime Water.—Into a 500-cc. volumetric flask was weighed 97.06 g. of 3-methylglucose and the flask filled to the mark with clear lime water saturated at 35° (0.0407 N).   The normality fell to 0.035.   The solution was molar with respect to 3-methylglucose and of a density 1.06 at 35°.   The flask was placed in a thermostat at 35° and the specific rotation and the aldohexose content determined.   These gradually decreased until at the end of about forty hours a constant value was reached.   The optical observations were made in a 2-dcm. jacketed tube at 20° on a 5% solution made acid to methyl orange.   The equilibrium rotation was 15.6 at the end of 288 hours and the aldohexose content 64.1%.   The methoxyl content was found to be 15.1% against a theoretical of 15.97%.   The P_H was 6.35 as determined by the quinhydrone electrode.   The polarimetric data are recorded in Table III together with the specific rotation calculated from the iodimetric data, assuming that 3-methylglucose and 3-methylfructose are the only substances present.

The tabular data are shown graphically in Fig. 3.   The close relation between the polarimetric data and the iodimetric data indicates that the main substances present in the reacting mixture at any time are 3-methylglucose and 3-methylfructose.

TABLE III

POLARIMETRIC DATA OF MOLAR 3-METHYLGLUCOSE IN 0.035 N CALCIUM
HYDROXIDE SOLUTION AT 35°

Time, hours	Observed α	$[\alpha]_D^{20}$	$[\alpha]_D^{20}$ Calculated from iodimetric data
. . .	. .	55.0	. .
12	2.53	25.5	27.5
17	2.36	23.2	24.1
34	1.82	17.32	16.8
47	1.59	15.9	16.8
59	1.67	16.0	15.9
288	1.63	15.6	15.9

TABLE IV

IODIMETRIC DATA OF MOLAR 3-METHYLGLUCOSE IN 0.035 N CALCIUM HYDROXIDE
SOLUTION

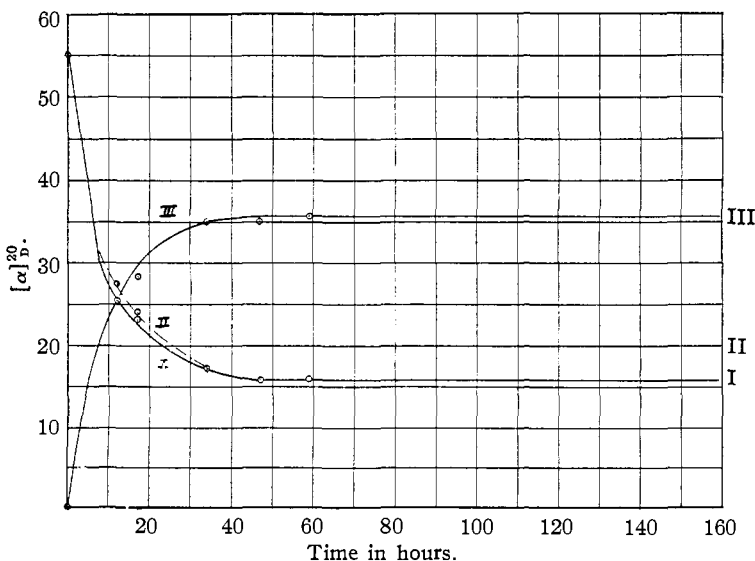| Time, hours	Aldose, %	Ketose (by difference), %	$[\alpha]_D^{20}$ Calculated from iodimetric data
12	74.8	25.2	27.5
17	71.6	28.4	24.1
34	65.0	35.0	16.8
47	64.9	35.1	16.8
59	64.1	35.9	15.9
288	64.1	35.9	15.9

Fig. 3.—Interconversion of 3-methylglucose in dilute alkali: I, observed
change in rotation; II, calculated change in rotation; III, percentage of 3-
methylglucose transformed into 3-methylfructose.

The fall in P_H indicated the formation of saccharinic acids. These were estimated as follows. To 51.81 g. of equilibrated solution, the original normality of which was 0.0351, was added the calculated amount of oxalic acid. The acids liberated corresponded to 3.46% of the original sugar transformed into saccharinic acid. If the calcium hydroxide originally present had all been neutralized by saccharinic acid formation, in accordance with the drop in P_H, the corresponding percentage of 3-methylglucose so transformed would have been 3.68%. Also, the loss of methoxyl corresponds roughly to the percentage of saccharinic acid found. Methyl alcohol was confirmed in the distillate.

In his study of the action of lime water on glucose at 35°, Wolfrom[2] isolated a small amount of mannose as the phenylhydrazone. In the present study, 3-methylmannose could not be identified, although the polarimetric and iodimetric data indicated that it should be present in small amounts. Moreover, the close resemblance in other respects between the action of lime water upon glucose and upon monomethylglucose supports this conclusion. The search was made more difficult by the unknown nature of 3-methylmannose. It seems reasonable to suppose that the specific rotation of 3-methylmannose would be close to that of mannose, or about 15°. The fact that the observed rotation of the equilibrated solution checked closely the rotation calculated from iodimetric data, assuming that only 3-methylglucose and 3-methylfructose were present, indicates that another optical body must have been present to offset the small amount of saccharinic acids present.

Separation of the Reaction Mixture.—In a 1-liter florence flask was placed 166 g. of the equilibrated solution containing 30.4 g. of products from which the lime had been removed as calcium oxalate. From the preliminary data the mixture, in terms of original sugar, contained about 64% of monomethylglucose and mannose, 32% of monomethyl ketoses and 3.5% of saccharinic acids. It was oxidized by the method of Hudson and Isbell[14] and the aqueous solution concentrated under diminished pressure to a volume of about 500 cc. To the cold solution was added gradually a suspension of freshly prepared pure calcium hydroxide until the neutral point of phenolphthalein was reached, then a clear solution of lime water was added slowly and the solution kept slightly alkaline for half an hour. After the addition of a drop of acetic acid to the solution, it was evaporated under diminished pressure to a volume of 30 cc. To the residue was added 500 cc. of methyl alcohol with vigorous mechanical stirring, then 400 cc. of acetone in the same manner. The calcium salts came out as a finely divided precipitate. The mixture was stirred rapidly for one hour longer. The lime salt was then filtered with suction onto a weighed filter, washed with methyl alcohol and dried *in vacuo* over sulfuric acid; yield, 22.7 g., or 94% of the theoretical 22.9 g. of calcium hexonate and 1.07 g. of calcium saccharinate. The salt, which showed a very slight reducing action toward Fehling's solution, was dissolved in 25 cc. of water in a 1-liter flask and reprecipitated with 500 cc. of methyl alcohol and 300 cc. of acetone, washed and dried; weight, 21.3 g., or 89% of the theoretical amount.

Anal. Calcd. for $(C_6H_{13}O_5COO)_2Ca$: Ca, 8.75. Found: Ca, 8.97.

Previous experience had shown the difficulty of avoiding basic salt formation under these conditions. A portion of the salt was fractionally precipitated, the first fraction showing 9.45% calcium and the second, 8.76. The second fraction showed $[\alpha]_D^{25} +12.9°$ ($\alpha = +0.99°$, 2.2 dcm., $c = 3.46$ in water).

In a 500-cc. flask was placed the remainder of the calcium salts, 17.85 g. To the hot solution of the salt in 200 cc. of water was added slowly a solution containing 4.91 g. of oxalic acid. The last of the calcium and oxalate ions was removed by adding small amounts of oxalic acid and calcium hydroxide until the solution showed the presence of both ions. The filtrate was evaporated under diminished pressure to a gum which

was then heated *in vacuo* at 95° for four hours; weight, 14.2 g., or 95% of the theoretical amount. The equivalent weight was 205 against a calcd. of 192 for 3-methylgluconic lactone; $[\alpha]_D^{25} + 46.1°$ initial and 31.8° final ($\alpha = +1.72°$, 2.2 dcm., $c = 2.45$ in water). A sample of acid gum from the oxidation of pure 3-methylglucose prepared in the same manner gave an equivalent weight of 206 and $[\alpha]_D^{25} +38.6°$ ($\alpha = +0.78°$, 1 dcm., $c = 2.03$ in water). The remainder of the gum, 13.0 g., was dissolved in 100 cc. of water and heated on the water-bath for four hours with 30 g. of recrystallized brucine. The resulting solution was extracted four times with 150-cc. portions of benzene, and the aqueous solution treated with decolorizing charcoal, filtered and evaporated under diminished pressure to a volume of about 50 cc. To this residue was then added 100 cc. of absolute alcohol. The mixture was allowed to crystallize in the refrigerator overnight. The crystals were filtered, washed thoroughly with absolute alcohol and dried; weight, 24.3 g., or 40.3% of the theoretical amount and melting at 148–150° when rapidly heated. A portion was recrystallized from dilute alcohol, and dried *in vacuo* over phosphorus pentoxide. It melted at 146–148° when heated from 135° at 3° per minute. The melting point of pure brucine 3-methylgluconate is 146–148°. There was no change in melting point on mixing the two; $[\alpha]_D^{25} -16.9°$ ($\alpha = -1.55°$, 2.2 dcm., $c = 4.16$ in water). The specific rotation of known brucine 3-methylgluconate under the same conditions was −16.7°. From the filtrate there was obtained with some difficulty 3.0 g. more of brucine salt, by adding slowly absolute alcohol and then acetone to the concentrated aqueous solution of the salt. The filtrate from this last treatment could not be induced to crystallize. It was, accordingly, converted to the cinchonine salt as follows. The residue was taken up in about 300 cc. of water and the brucine precipitated by adding a considerable excess of barium hydroxide. The dried brucine from the solution weighed 8.7 g. The alkaline filtrate was extracted three times with benzene and the barium precipitated from the aqueous extract as completely as possible with dilute sulfuric acid. The filtrate was heated on the steam-bath with 6.0 g. of pure cinchonine for ten hours with rapid mechanical stirring. The solution took up 4.3 g. of cinchonine. The filtrate was evaporated under diminished pressure to a sirup which was taken up in absolute alcohol and the evaporation repeated. The final residue was dissolved in 10 cc. of absolute alcohol and allowed to crystallize in the refrigerator. The product was filtered and washed with acetone; weight, 3.2 g. This was converted back to the brucine salt. There was obtained in this way, 3.2 g. more of pure brucine 3-methylgluconate, melting at 146–148°. There was obtained from the cinchonine salt mother liquor, 1.1 g. more cinchonine salt. This was also converted back to the brucine salt; weight, 0.9 g., melting at 140–142°. The second crop of impure brucine salt (3.0 g.) was dissolved in alcohol, the solution decolorized and reduced to a small volume. Failing to obtain crystals, the salt was thrown out by the addition of acetone; weight of dried salt, 1.5 g., melting at 125–135°. The final cinchonine salt residue was converted back to the brucine salt. An acid gum weighing 1.0 g. was obtained from these cinchonine salt residues. The brucine salt was very soluble in alcohol and was only partially thrown out by addition of acetone; weight of brucine salt, 1.7 g. All the filtrates from the brucine salt residues were combined and evaporated to dryness; weight of final brucine salt residue, 3.1 g., melting at 70–75°. The total weight of pure brucine 3-methylgluconate was 27.5 g., or 72% of the theoretical amount, based on the gum taken. In addition, there was a total of 7.2 g. of impure brucine salts. The total weight of brucine salts was 34.7 g., or 91% of the theoretical, based on the weight of the gum taken. Of the brucine salt residues, 6.3 g. was converted back to the acid gum, which was dried *in vacuo* over sulfuric acid at 80° for four hours. This gum showed an equivalent weight of 196 against the calculated 192 for 3-methylgluconic

lactone; methoxyl 10.7 against 16.15; $[\alpha]_D^{25}$ +53.5° initial and +34° final (α = +0.40°, 1 dcm., c = 1.18 in water). These data indicate a mixture of demethylated acids believed to be saccharinic and methylhexonic.

The methyl alcohol–acetone filtrate from the lime salts containing the ketoses was evaporated under diminished pressure to a sirup. About 20 cc. of acetone was added and the solution again evaporated. This was repeated once more. The final sirup was nucleated with a crystal of 3-methylfructose and placed in the refrigerator overnight. Ether was then added a little at a time until the total volume was about 300 cc. The mixture was then filtered and the sugar dried, first in the air and then *in vacuo*, over sulfuric acid; weight, 8.5 g. The filtrate was concentrated and gave 0.45 g. more sugar, bringing the total yield up to 9.0 g. From the analytical data the theoretical yield of ketoses should be 9.86 g. The weight is, therefore, 91% of the theoretical amount.

From a sample of the ketose isolated from the equilibrated solution in the above way, was isolated α-diacetone-3-methylfructose identical in properties with that obtained by methylating α-diacetone-fructose. To this end the ketose was dissolved in acetone containing five drops of concentrated sulfuric acid in 100 cc. of solution and the mixture allowed to stand for four hours. The acid was neutralized with ammonia gas, the solution filtered and evaporated under diminished pressure. The crystalline residue was dissolved in 100 cc. of ether, the ether solution washed once with water, dried with anhydrous potassium carbonate, and reduced to 5 cc., whereupon the characteristic crystals of α-diacetone-3-methylfructose separated. The washed, dried crystals melted at 114–115°; recorded, 114–115°.[18]

The osazone was prepared and recrystallized several times from water–alcohol mixtures. It melted at 175–177° and did not change on mixing with known 3-methylfructose osazone; recorded, 177–179°.[16] The final sugar residue which would not crystallize was recovered. It weighed 1.5 g. after thorough drying, $[\alpha]_D^{25}$ +12° (α = +0.15°, 1 dcm., c = 2.45 in water). Only oily products resulted on attempting to convert the material to an osazone. The change of specific rotation of the sugar from the mixture with temperature agreed very well with that shown by pure 3-methylfructose.

TABLE V

COMPARISON OF CHANGE WITH TEMPERATURE OF SPECIFIC ROTATION OF SUGAR ISOLATED WITH THAT OF PURE 3-METHYLFRUCTOSE

Temp., °C	$[\alpha]_D$ of sugar from mixture	$[\alpha]_D$ 3-Methylfructose
20	−53.9	−53.5
38	−43.0	−42.7
45	−40.0	−38.5

A second attempt was made to separate the constituents of the mixture. In this experiment the procedure was exactly the same except that the acid was converted to the calcium salt by the use of calcium carbonate instead of calcium hydroxide. From 166 g. of the equilibrated solution containing originally 30.4 g. of 3-methylglucose and having a final composition of 64.1% of 3-methyl aldoses, 3.5% of saccharinic acid and 32.4% 3-methyl ketoses, in terms of the original sugar, there was obtained 20.9 g. of lime salts out of the theoretical 23.97 g., or 87% of the theoretical amount, and 9.0 g. of 3-methylfructose, $[\alpha]_D^{20}$ −54.5° (α = −5.33°, 2 dcm., c = 4.89) out of the theoretical 9.86 g., or 91% of the theoretical amount.

The residue from the isolation of 3-methylfructose was evaporated under diminished

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[16] Ref. 11, p. 1337.

pressure to a gum; weight, 3.5 g.   This gum was acidic, 0.2271 g. neutralized 0.394 cc. of N base.   The same weight (0.2271 g.) of 3-methylgluconic acid would neutralize 1.08 cc. of N base.   This residue was free from inorganic material.   The remainder of the residue, 3.3 g., was dissolved in 25 cc. of water and heated on the steam-bath with excess calcium carbonate.   From the filtrate was obtained in the usual way 0.8 g. of calcium salt.   The final sugar residue could not be induced to crystallize.

The isolation of the crude calcium 3-methylgluconate and pure 3-methylfructose in high yields served as a useful check on the polarimetric and iodimetric data.   The properties of the calcium salt made it necessary to employ the alkaloidal salts for the isolation of the pure 3-methylgluconic acid derivative.   The data obtained from the separation of the products from the mixture check with the conclusions reached from analytical data that the principal constituents of the equilibrated solution are 3-methylglucose and 3-methylfructose.

Behavior of 3-Methylfructose in Lime Water Saturated at 35°.—While it was known from the work of Wolfrom[2] and Greene[3] that molar solutions of glucose and fructose in 0.035 N calcium hydroxide solution give very different final specific rotations when allowed to stand at 35°, it was hoped that 3-methylglucose and 3-methylfructose, on account of their much greater speed of conversion, would reach the same final rotation, thus evidencing a true equilibrium.   This was, however, not the case.   The fact that 3-methylglucose and 3-methylfructose do not give the same equilibrium rotation makes the investigation of the latter a separate problem and demonstrates clearly that in the case of the monomethyl sugars the Lobry de Bruyn interconversion in lime water does not give rise to a true equilibrium.

The change of rotation of a molar solution of 3-methylfructose in 0.035 N calcium hydroxide solution kept at 35° is shown in the following table.

TABLE VI

POLARIMETRIC DATA OF MOLAR 3-METHYLFRUCTOSE IN 0.035 N CALCIUM HYDROXIDE SOLUTION

Time, hours	α	$\alpha]_D^{30 \, a}$
0	....	−53.0
16	−5.49	−27.8
21.5	−5.08	−26.2
29.5	−4.25	−21.9
48.5	−4.25	−21.9

[a] (1 dcm., c = 19.4 in water.)

The equilibrium rotation corresponds to about 29% of 3-methylglucose, assuming that the only products are 3-methylglucose and 3-methylfructose.   The equilibrium rotation of molar 3-methylglucose in lime water was +15.9°.

Summary

1.   The conversion of 3-methylglucose into 3-methylfructose with lime water at 35° confirms the recorded position of the methyl group in 3-methylfructose.

2.   Experimental evidence is given in support of the enediol theory and the precise blocking of enolization by methyl groups.

3.   The enediol of 3-methylglucose is shown to resemble that of glucose rather than that of tetramethylglucose.

4.   The absence of a high iodine absorbing product in the reaction mixture when 3-methylglucose is treated with lime water at 35° is taken as evidence that the 2,3-monomethyl-enediol does not form.

5.   When 3-methylglucose is treated with lime water at 35° to no further change, about 64% of the sugar consists of aldose and 32% is converted to 3-methylfructose.   Indirect evidence is given for the formation of about 3.0% of saccharinic acid.   The remainder consists of a mixture of partially demethylated hexoses.

6.   At 35° in dilute alkali the methyl group in 3-methylglucose is slowly removed as methyl alcohol.   At 100° under the same conditions the loss is rapid.

EVANSTON, ILLINOIS

A STUDY OF THE HYDROLYSIS OF CORN STARCH AND ITS AMYLOSES WITH REFERENCE TO THE PRODUCTION OF GENTIOBIOSE[1]

BY T. C. TAYLOR AND DAVID LIFSCHITZ

When the starches are hydrolyzed by means of dilute acid or by enzymes the disaccharide, maltose may be isolated.[2]   Its concentration in the solution passes through a maximum and then decreases.[3]   With enzymes it is the only disaccharide that is produced and comes apparently directly by hydrolytic scission.   For this and other reasons the maltose residue is considered to be a part of the carbohydrate structure of the starches.

Another diglucose sugar, however, has been identified recently in the solution from the hydrolysis of corn starch.[4]   This sugar, gentiobiose, is a reducing β-glucoside, the link being at position 6 in contrast to maltose which is an α-glucoside and linked at position 4.   As isolated both are delta lactoles.

It is known that in the presence of mineral acids glucose will undergo condensation to form amorphous uncharacterized polysaccharides.   The first such synthesis was reported by Fischer.[5]   It is carried out by allowing a 20% solution of glucose in *concentrated hydrochloric acid* (37%) to stand for *fifteen hours to three days* at *room temperature*, or *lower*, and at atmos-

[2] (a) For bibliography see Walton, "A Comprehensive Survey of Starch Chemistry," New York, 1928;   (b) Lintner and Düll, *Ber.*, **28**, 1522 (1895);   (c) v. Friedrichs, *Arkiv Kemi, Min. Geol.*, **5**, No. 2 (1913–1915).

[3] Rolfe and Defren, THIS JOURNAL, **18**, 869 (1896).

[4] Berlin, *ibid.*, **48**, 2627 (1926).

[5] Fischer, *Ber.*, **23**, 3687 (1890);   **28**, 3204 (1895).