however, a band with relatively low intensity, at frequency number 3628, yielding a separation of 98 mm.$^{-1}$. As this band is sharply defined and easily measured, there is no doubt as to its exact location. It is possible that by using greater thicknesses of solid benzene at the low temperature this band might be observed and its position checked under those conditions. The data for toluene, when tabulated in a similar manner, show an average separation of 95 mm.$^{-1}$, which compares favorably with that of 94.3 mm.$^{-1}$, found by Klingstedt for a hexane solution.

Since the above article was submitted for publication, a correction by Smith, Boord, Adams and Pease to their previous article[9] has appeared in This Journal, **49**, 3137 (1927).

Summary

1. The absorption curves of benzene and toluene in absolute ethyl alcohol solution have been determined and their relations discussed.

2. An analysis of their spectra shows that with one possible exception all of the observed bands of benzene and toluene can be arranged in series, in accordance with a simple mathematical expression.

3. The results recently reported by Smith, Boord, Adams and Pease indicating a benzene-like structure for the absorption spectra of diethyl ether, methyl-*n*-amyl ether, cyclohexene and ethylene chlorohydrin have been proved to be due to the presence of benzene as an impurity in these compounds.

Ithaca, New York

[Contribution from the Chemical Laboratory of Northwestern University]

THE REACTIVITY OF THE METHYLATED SUGARS. II. THE ACTION OF DILUTE ALKALI ON TETRAMETHYL GLUCOSE

By M. L. Wolfrom[1] with W. Lee Lewis

Received November 3, 1927 Published March 7, 1928

The conversion of one sugar into another and the chemistry of sugars in alkaline media are matters of interest to the biologist and pathologist, as well as the chemist. The course of these transformations can never be fixed with exactness until the underlying fundamental chemistry has been elucidated. The interconversion of simple sugars by alkali has been explained by assuming intermediate enol formation, but no direct proof of this has been obtained. Thus, the reciprocal interconversion of *d*-glucose, *d*-mannose and *d*-fructose, discovered by Lobry de Bruyn and

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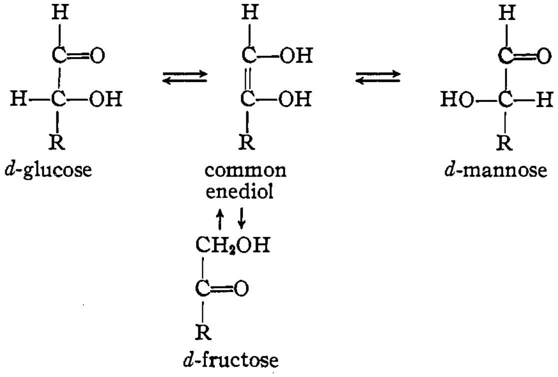
[1] Abstracted from a dissertation submitted by M. L. Wolfrom to the Graduate School of Northwestern University, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

van Ekenstein,[2] is explained[3] by assuming an enediol common to the three sugars.



Sugar enol formation and interconversion were explained by Nef[4] on the basis of selective hydration and dehydration. This has been shown[5] to be incompatible with the behavior of the methylated sugars, as they would thus form hemi-acetals which would probably lose methyl alcohol almost as readily as the hypothetical hydrates of the non-methylated sugars would lose water. The experimental facts may be harmonized by replacing this conception of selective hydration and dehydration by a simple assumption of keto-enol tautomerism, due solely to the migration of a hydrogen atom.

The changes depicted in the formulas above represent the first steps in the action of dilute alkali on *d*-glucose. By increasing the time, temperature and concentration of alkali, more deep-seated changes take place. Enolization may progress down the carbon chain with the formation of other ketoses. That many of these more deep-seated changes occurred in the experimental work of Lobry de Bruyn and van Ekenstein and greatly complicated their results is evident from their own statements.[6]

If the previously described common enediol explanation of the mutual interconversion of *d*-glucose, *d*-mannose and *d*-fructose is correct, then on applying this to the case of tetramethyl glucose, certain predictions may at once be made. In this derivative one of the labile hydrogen atoms is replaced by a stable methyl group and, consequently, only the 1,2-monomethyl enediol can form. This could not undergo all the changes possible for the unsubstituted enediol, since the methyl group of the methoxyl could not migrate in the same way, presumably, as the hydrogen of an hydroxyl. Thus the possible changes are as follows.

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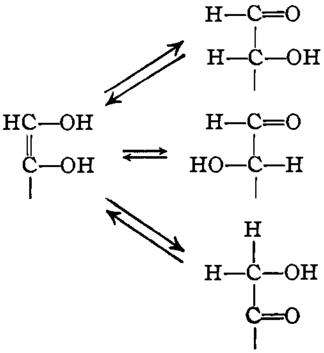
[2] Lobry de Bruyn and van Ekenstein, *Rec. trav. chim.*, **14,** 195–207 (1895).

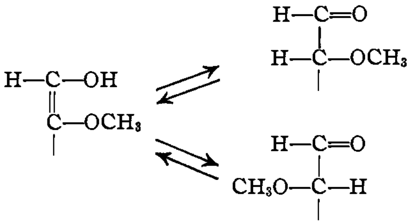
[3] Wohl and Neuberg, *Ber.*, **33,** 3099 (1900).

[4] (a) Nef, *Ann.*, **357,** 295 (1907); (b) Spoehr, *Am. Chem. J.*, **43,** 228 (1910).

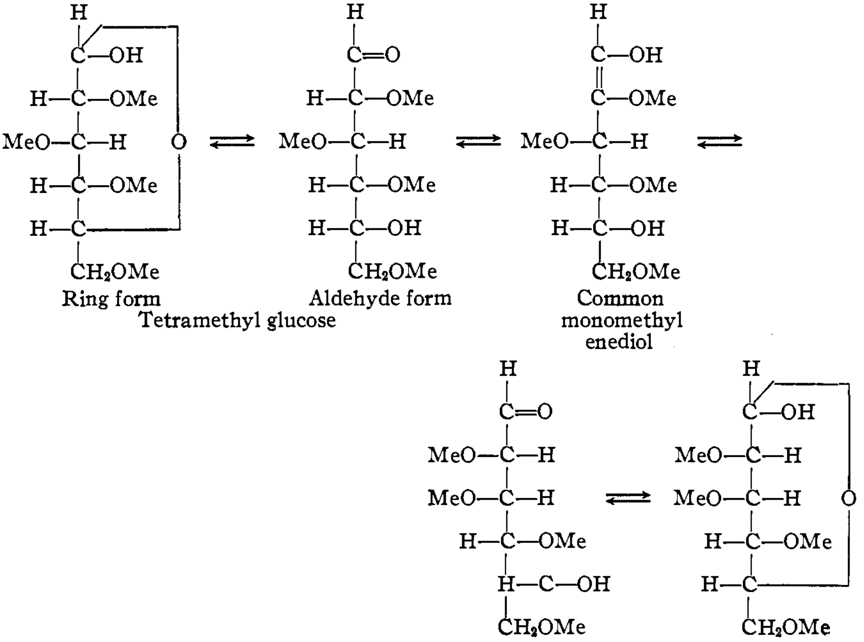
[5] Gustus and Lewis, THIS JOURNAL, **49,** 1513 (1927).

[6] Lobry de Bruyn and van Ekenstein, *Rec. trav. chim.*, **15,** 93 (1896).





On the basis of these assumptions it would be expected that tetramethyl glucose would be converted by alkali into tetramethyl mannose but not into a methylated fructose. These changes may be formulated as follows.



Ring form Aldehyde form

Tetramethyl glucose

Common monomethyl enediol

Aldehyde form Ring form

Tetramethyl mannose

The existence of the aldehyde form of tetramethyl glucose has been proved by Levene and Meyer[7] through isolation of the open chain pentamethyl glucose. In the above formulas we have depicted tetramethyl glucose with the 1,5 oxidic ring in accordance with the most generally accepted evidence, although in our work we are not concerned with the absolute ether linkage.

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[7] Levene and Meyer, *J. Biol. Chem.*, **69**, 175 (1926).

In the unmethylated sugars interconvertibility does not demand that the final sugar have the same oxidic ring as its source. Methylation should fix the position of the oxidic ring so that two tetramethyl aldohexoses which are interconvertible should possess the same oxidic ring, whatever that may be.

In testing the deductions of the common enediol theory developed above, it was found necessary to establish first better interconversion conditions for *d*-glucose than are recorded in the literature. In the work of Lobry de Bruyn and van Ekenstein simultaneous saccharinic acid formation to the extent of 20 to 30% of the sugar used is recorded.[8] In this work saccharinic acid formation and the formation of ketoses other than fructose were finally restricted to a negligible amount by using extremely mild conditions. *d*-Glucose was converted by dilute alkali into a mixture of *d*-glucose, *d*-fructose and *d*-mannose. The amount of each sugar present was determined and it was found that under the conditions used, 31% of the original glucose was changed to fructose, 2.5% to mannose, while 63.5% remained unchanged.

The interconversion conditions established for glucose were then applied to tetramethyl glucose. On treatment of the equilibrated solution from tetramethyl glucose with acid it was proved by iodimetric methods that all of the original sugar was present as tetramethyl aldohexose, no ketose being present. The specific rotation of the resultant sugar mixture, +42 to +43°, was that required for an equal mixture of tetramethyl glucose, $[\alpha]_D = +82°$, and tetramethyl mannose, $[\alpha]_D = +2.4°$. The tetramethyl mannose used in this work was crystalline material.[9] The same constant specific rotation was reached on treating tetramethyl mannose[10] under the same conditions and a synthetic mixture of equal parts of tetramethyl glucose and tetramethyl mannose maintained a stable rotation when treated with alkali. Solutions of tetramethyl glucose on treatment with different concentrations of dilute alkali showed widely differing initial polarimetric readings but all reached the value for the equimolecular mixture, the relatively large amount of tetramethyl mannose formed from tetramethyl glucose contrasting sharply with the behavior of the unmethylated glucose.

This sirupy mixture gave a high yield of crystalline anilide,[11] which together with the other analytical data indicates the presence of only negligible quantities of substances, other than the two epimers. Acids and methyl alcohol are not formed. The free sugars were recovered.

The abnormally high iodine value of the mixture dropped on acidifi-

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[8] Ref. 2, pp. 198–199.

[9] Lewis and Greene, *Science*, **64**, 206 (1926).

[10] Unpublished work of R. D. Greene of this Laboratory.

[11] (a) Irvine, *J. Chem. Soc.*, **93**, 103 (1908); (b) Irvine and McNicoll, *ibid.*, **97**, 1452 (1910).

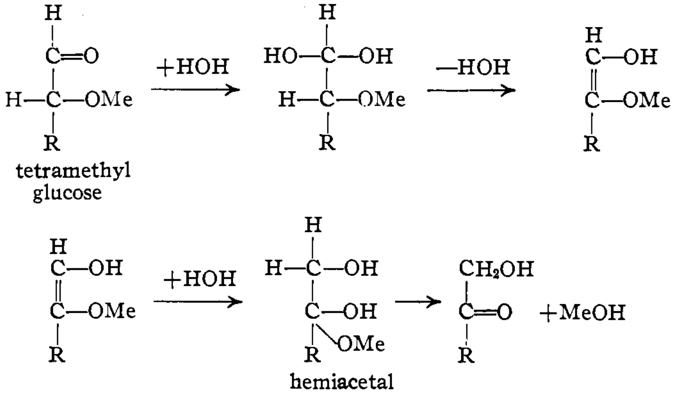
cation to the normal figure for a tetramethyl aldohexose solution, thus indicating the existence of a stable monomethyl enediol capable of consuming considerably more iodine.[12] Increasing the acid content did not alter the value.

These results were not shown by the unmethylated sugars, probably due to the decrease in stability of the enediols,[10] a point which is to be further investigated.

The experimental results obtained thus confirm the theory of a common enediol transformation in alkaline solvents, this in presence of acid yielding equal quantities of methylated glucose and mannose.

The bearing of this on the important controversial question of ring structure[13] of carbohydrates is also of particular interest. We have found that the so-called "normal" forms of tetramethyl glucose and tetramethyl mannose, prepared by methylation of the naturally occurring sugars, are mutually interconvertible and must, therefore, possess the *same* oxidic ring structure.[14]

From the experimental work some conclusions may also be drawn concerning the mechanism of keto-enol tautomerism in general. If, as is sometimes assumed, enol formation takes place through selective hydration and dehydration, then in the case of tetramethyl glucose hemiacetals would be formed. These, as previously noted, would readily lose methyl alcohol with the formation of partially methylated ketoses.



In this work no ketones and no methyl alcohol were found and, consequently, the selective hydration and dehydration theory of enediol formation is untenable at least in this instance. On the basis of our organic

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[12] Wislicenus and Thoma, *Ann.*, **436**, 43, 45 (1924).

[13] (a) Hudson, THIS JOURNAL, **48**, 1424 (1926); (b) Levene and Simms, *J. Biol. Chem.*, **68**, 737 (1926); (c) Charlton, Haworth and Peat, *J. Chem. Soc.*, **128**, 82 (1926); (d) Hirst, *ibid.*, **128**, 350 (1926).

[14] See, however, Dale, THIS JOURNAL, **46**, 1046 (1924); Hudson, *ibid.*, **48**, 1433 (1926).

structural formulas the change is represented by a shift in the attachment of hydrogen between a carbon and oxygen atom, the mechanism of this shift not being one of chemical hydration and dehydration. It is conceivable that this deduction may be extended to the general theory of keto-enol tautomerism.

Experimental Part

Conversion of Glucose into Mannose and Fructose with Minimum Secondary Changes.—A molar solution of *d*-glucose in clear lime water saturated at 35° attained polarimetric equilibrium on standing for ten days at that temperature. Xylene was used as antiseptic agent. The initial acidimetric normality of the sugar solution was 0.035. The initial *P*H of the lime water alone was 12.6, which dropped to 10.6 on addition of the sugar. The solution very slowly changed to neutral and on standing for over a year had not developed any free acidity. The polarimetric changes are given in the following table and are plotted in Fig. 1.

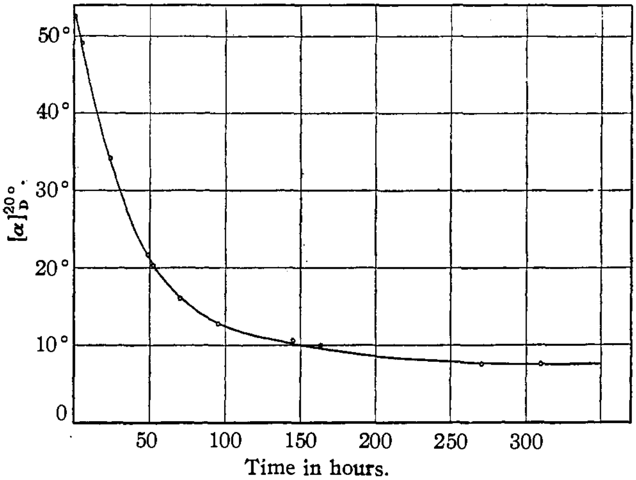

Fig. 1.—Polarimetric change of *d*-glucose in dilute alkali.

Table I

Polarimetric Change of *d*-Glucose in Dilute Alkali

Time, hours	α, 20°, 2 dcm. tube, molar soln.	$[\alpha]_D^{20°}$	Time, hours	α, 20°, 2 dcm. tube, molar soln.	$[\alpha]_D^{20°}$
0.5	+18.85°	+52.3°	94	4.61°	12.8°
1	18.80°	52.2°	144	3.88°	10.8°
5.5	17.78°	49.3°	163	3.54°	9.8°
23	12.31°	34.2°	270	2.7°	7.5°
48.5	7.78°	21.6°	310	2.7°	7.5°
53	7.36°	20.4°	25 days	2.7°	7.5°
70	5.81°	16.1°			

The equilibrated mixture was analyzed. The total reducing sugar content was determined by the Munson-Walker method[15] and corrected for the reducing power of the sugars concerned. The aldohexose content was determined by iodine oxidation according to the method of Cajori[16] modified for the presence of mannose by lengthening the time of oxidation to one hour. In both of these analyses suitable samples of the solutions were obtained by means of a weight pipet. The amount of mannose present was found, after concentration of the neutral solution to half volume, by quantitative precipitation as the phenylhydrazone under conditions precluding glucosazone formation, *viz.*, 5°.[17] These methods were checked in this Laboratory[10] with solutions of glucose, mannose and fructose of known composition and found to be accurate. The data determined follow.

TABLE II

ANALYSIS OF SUGAR SOLUTION FROM *d*-GLUCOSE

Original sugar content	16.83% by wt. soln.
Final reducing sugar content (calculated as glucose)	15.9%
Aldohexose content	11.07%
Mannose content	0.40%

The final analysis of transformed glucose calculated from the preceding data is given in Table III.

TABLE III

COMPOSITION OF SUGAR MIXTURE FROM *d*-GLUCOSE

Glucose	63.4%	Fructose	30.9%
Mannose	2.4%	Non-sugar substances, probably saccharinic acids	3.3%

The final polarimetric reading calculated from the sugars in Table III would be +5, whereas +2.7 was the observed value.

These data show that no appreciable amount of ketoses other than fructose could be present as it is known that mixtures of these unfermentable ketoses have very low reducing properties (about half that of glucose) and slight optical activity.[18]

The rate of formation of fructose may be calculated with a fair degree of accuracy from the optical data since the change in rotation is indicative of the transformation of glucose into fructose. These data are plotted in Fig. 2 and are tabulated below.

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[15] Munson and Walker, THIS JOURNAL, **28**, 663 (1906).

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

[17] Sherrard and Blanco, *Ind. Eng. Chem.*, **15**, 612 (1923).

[18] (a) Lobry de Bruyn and van Ekenstein, *Rec. trav. chim.*, **16**, 256–263 (1897); (b) Dakin and Dudley, *J. Biol. Chem.*, **15**, 127 (1913); (c) Spoehr and Wilbur, *ibid.*, **69**, 421 (1926).

TABLE IV

RATE OF TRANSFORMATION OF GLUCOSE INTO FRUCTOSE

Time, hours	Specific rotation	Original glucose converted into fructose, %
5.5	+49.3°	2.0
23	34.2	12.5
48.5	21.6	21.2
53	20.4	22.0
70	16.1	25.0
94	12.8	27.3
144	10.8	28.7
Final	7.5	31.0

Final value determined by analysis, + 30.9%.

Preparation of Methylated Sugars Used.—Four hundred and fifty g. of pure, twice recrystallized tetramethyl glucose, normal form (m. p. 88°; $[\alpha]_D^{20}$ = +82°, *c* = molar) was prepared according to the method of W. N. Haworth,[19] suitably modified for the handling of large quantities of material. Four and four-tenths g. of tetramethyl fructose, normal form (m. p. 92–93°), was prepared in pure condition according to the method of Irvine and of Hudson.[20] Thirty-six g. of tetramethyl mannose, normal form, was prepared according to the method of W. N. Haworth.[21] The sirup obtained by this method crystallized on nucleation with crystalline material obtained by R. D. Greene of this Laboratory[9] and was purified by recrystallization from low-boiling petroleum ether, m. p. 50–51°; $[\alpha]_D^{20}$ = +27.5° in methyl alcohol, *c* = 5.000.

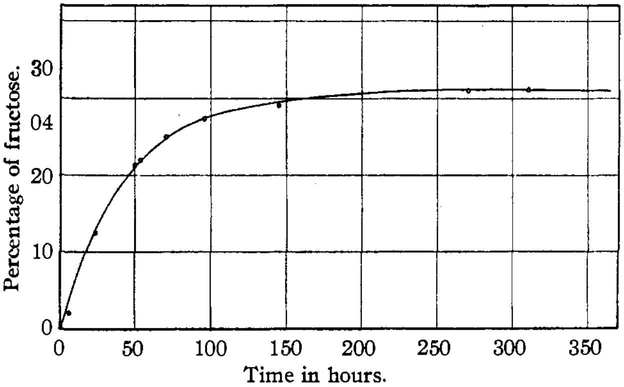

Fig. 2.—Transformation of glucose into fructose.

Application of Established Interconversion Conditions to Tetramethyl Glucose.—A molar solution of tetramethyl glucose in 0.035 normal calcium hydroxide solution changed in specific rotation from +82° to 42° (constant) on standing at 35° for 150 hours. The solution gradually became neutral and showed no acid reaction after standing at room temperature for several months. The equilibrated mixture was examined, as reported below, for the presence of ketoses, methyl alcohol and acids,

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[19] Haworth, *J. Chem. Soc.*, **107**, 8 (1915).

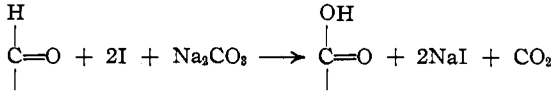
[20] (a) Irvine and Patterson, *ibid.*, **121**, 2696 (1922); (b) Hudson and Brauns, THIS JOURNAL, **38**, 1216 (1916).

[21] Ref. 19, p. 13.

after which its composition was established by optical data, and by separation of its components as anilides.

Proof of Absence of Ketoses in Equilibrated Mixtures from Tetramethyl Glucose.—Iodimetry is applied to the unmethylated hexose sugars to determine aldohexoses and thus ketohexoses by difference. Proper conditions were found in our work for the application of this method to the methylated sugars concerned. Attempts to use copper reducing values for determining tetramethyl aldohexose sugars were unsuccessful as the accuracy of the methods when applied to the methylated sugars is inadequate. The procedure adopted for the determination of methylated aldohexoses by iodimetry follows.

To 20 cc. of solution containing approximately 0.1 g. of methylated sugar in a glass stoppered flask, was pipetted 25 cc. of 0.1 *N* iodine solution. Four cc. of 2.75 normal sodium carbonate solution was then added. The mixture was allowed to stand for two to three hours at room temperature and in the dark. The sodium carbonate was then neutralized with 10% sulfuric acid and about 1.5 cc. of acid added in excess. The iodine remaining was titrated with 0.05 *N* sodium thiosulfate solution and the amount of iodine consumed calculated. With molar sugar solutions the sample was obtained by weighing the appropriate amount (0.5 cc.) of solution of determined density. Addition of iodine to the diluted sugar solution is essential as is also the order of addition of the iodine and carbonate. Under these conditions the theoretical amount of iodine was consumed according to the following equation.



$$\overset{\displaystyle H}{\underset{|}{\overset{|}{C}}}{=}O + 2I + Na_2CO_3 \longrightarrow \overset{\displaystyle OH}{\underset{|}{\overset{|}{C}}}{=}O + 2NaI + CO_2$$

Careful control work showed that neither the sodium carbonate nor the small amount of alkali introduced from the sugar solution interfered with the procedure. Variation in the room temperature (17 to 24°) also had no detectable effect. Oxidation was complete in two hours although another hour did not alter the figure beyond the limit of error. The oxidation of tetramethyl mannose was not complete at one hour. The data of Table V show that tetramethyl fructose is not oxidized under the conditions of the experiment.

TABLE V

STABILITY OF TETRAMETHYL FRUCTOSE TOWARD IODINE OXIDATION

	a	b
Tetramethyl fructose, g	0.1000	0.1000
Iodine added, cc	25.00	25.00
Iodine unused, cc	24.95	24.95

The data of Table VI show that the procedure is applicable to mixtures of the two aldoses and in mixtures of the three sugars gives accurate results for tetramethyl fructose when the total sugar content is known.

Application of Established Iodimetric Method to Equilibrated Solution.—Application of this method to the equilibrated solution from tetramethyl glucose gave a value of 135–140% tetramethyl aldohexose. This was at first thought to be due to the reactive "gamma" form of tetramethyl mannose, but this form was not present, as the resulting

TABLE VI

IODIMETRIC ANALYSIS OF KNOWN MIXTURES OF METHYLATED SUGARS

Anal.		Tetramethyl glucose		Tetramethyl mannose		Tetramethyl aldohexose		Tetramethyl fructose	
		g.	%	g.	%	g.	%	g.	%
No. 1	Known	0.0581	57.8	0.0425	42.2	0.1006	100.0	....	...
	Found	....	..	....	..	.0991	98.7	....	...
No. 2	Known	.0585	59.1	.0405	40.9	.0990	100.0	....	...
	Found	....	..	....	..	.1002	101.0	....	...
No. 3	Known	.0609	60.4	.0399	39.6	.1008	100.0	....	...
	Found	....	..	....	..	.1005	99.7	....	...
No. 4	Known	.0593	58.5	.0420	41.5	.1013	100.0	....	...
	Found	....	..	....	..	.1016	100.4	....	...
No. 5	Known	.0500	45.2	.0210	18.9	.0710	64.2	0.0396	35.8
	Found	....	..	....	..	.0706	63.8	....	36.2[a]
No. 6	Known	.0500	45.2	.0210	18.9	.0710	64.2	.0396	35.8
	Found	....	..	....	..	.0709	64.1	....	35.9[a]

[a] By difference.

sirup did not condense[22] rapidly at room temperature with absolute methyl alcohol containing 0.25% of dry hydrogen chloride. The abnormal result could be accounted for if a relatively stable form of the common monomethyl-enediol was present since, due to its ethylenic nature, it would absorb halogen. If this were true, treatment with acid should revert the enol to the more stable keto form and the iodine value should be lowered. This was found to be the case and on increasing the acidification or "de-enolizing" conditions the constant value of 100% tetramethyl aldohexose was obtained.

TABLE VII

EQUILIBRATED MIXTURE FROM TETRAMETHYL GLUCOSE. CONSTANCY OF 100% APPARENT ALDOHEXOSE IODINE VALUE

Normality HCl	Time, standing, hours	Temp., °C.	Apparent tetramethyl aldohexose, %	
			Reaction mixture	Control[a]
.	..	..	137	...
1	0.5	20	113	...
1	24	20	105	99.4
2	36	35	100	100
3	54	35	99.4	100

Concentration of sugar in acid soln. = 0.5%.

[a] Pure methylated sugar solns. of known compositions.

For these acid solutions the iodimetric procedure was modified as follows.

The iodine was added to the acid solution and the acid then neutralized by dropwise addition of the previously determined amount of standard sodium hydroxide solution. The customary amount of sodium carbonate was then added and the solution allowed to stand, etc. Carbonate

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[22] Irvine and Burt, *J. Chem. Soc.*, **125**, 1347 (1924).

neutralization cannot be used as the carbon dioxide formed renders the resulting oxidation mixture too acid.

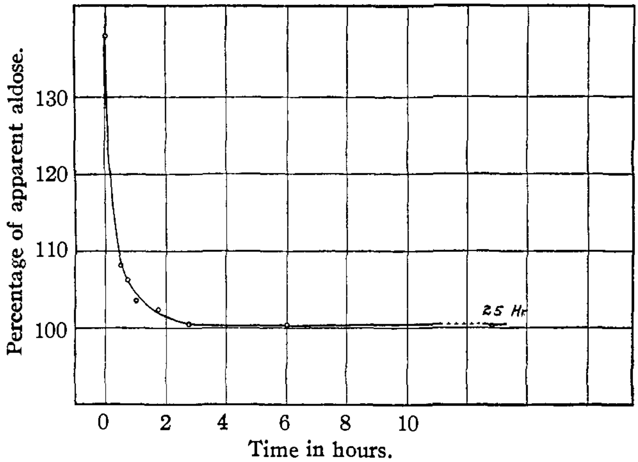

Fig. 3.—De-enolization of equilibrated mixture from tetramethyl glucose.

The rate of de-enolization of a more concentrated sugar solution was determined as follows and is plotted in Fig. 3. The conditions employed were one-fourth molar sugar solution in 3 *N* hydrochloric acid at 35°.

Table VIII

Rate of "De-Enolization" of Equilibrated Mixture from Tetramethyl Glucose

Time, hours	Tetramethyl aldohexose, apparent %	Time, hours	Tetramethyl aldohexose, apparent %
0	138	1.75	102.7
.5	108.1	2.75	100.3
.75	106.3	6.0	100.5
1.0	103.3	25.0	100.4

From the preceding data it is seen that the constant value of 100% tetramethyl aldohexose is obtained on treatment of the equilibrated solution with acid and that, therefore, no methylated ketoses are present.

Absence of Methyl Alcohol.—The equilibrated solution and the de-enolized solution were examined for methyl alcohol according to customary methods with negative results.

Absence of Acid Substances.—The absence of acid substances was demonstrated in three ways. The accuracy of methods (2) and (3) was sufficient to show that not more than 1% of the sugar could have been transformed into acid substances.

(1) The neutral, equilibrated molar sugar solution was initially 0.035 *N* alkaline and thus the maximum limit of 3.5% conversion of the sugar into acid is set. The calcium could, of course, disappear as alcoholate,

(2) By removal of the calcium from the equilibrated solution as oxalate and titration of resulting solution.

(3) By alkali titration (phenolphthalein indicator) of the de-enolized solution, the original acidity of which was known.

Composition of Resultant Tetramethyl Aldohexose Solution, Optical Data.—By iodimetric data we have shown that the solution from tetramethyl glucose, equilibrated with alkali and de-enolized with acid, contains 100% aldohexose sugar. The optical data in the two following tables constitute evidence that tetramethyl glucose, $[\alpha]_D = +82°$, and tetramethyl mannose, $[\alpha]_D = +2.4°$, are present in equal amounts. No appreciable difference in specific rotation was found before and after de-enolization. Solutions of tetramethyl glucose on treatment with different concentrations of dilute alkali show widely differing initial polarimetric readings, but all reach the value for the equimolecular mixture, +42 ± 1°. The same final specific rotation is reached on treating tetramethyl mannose under the same conditions and an equimolecular synthetic mixture of the two sugars maintains a stable specific rotation when treated with dilute alkali.

TABLE IX

POLARIMETRIC CHANGES OF METHYLATED GLUCOSE AND MANNOSE IN VARIOUS CONCENTRATIONS OF DILUTE ALKALI

Sugar	Normality of alkali	Time at 35°, hrs.	Observed reading	Time length	$[\alpha]^{20°}_D$
(*a*) Tetramethyl glucose	0.03 $Ca(OH)_2$	3	+37.39°	2 dcm.	+79.2°
		19	32.78	2 dcm.	69.4
		26	31.30	2 dcm.	66.3
		45	27.66	2 dcm.	58.6
		118	22.03	2 dcm.	46.6
		144	10.50	1 dcm.	44.5
		182	10.3	1 dcm.	43.6
(*b*) Tetramethyl glucose	0.04 $Ca(OH)_2$	7	35.65	2 dcm.	75.5
		29	29.25	2 dcm.	61.9
		50	26.00	2 dcm.	55.0
		70	24.05	2 dcm.	50.9
		96	22.75	2 dcm.	48.2
		121	21.85	2 dcm.	46.2
		169	20.87	2 dcm.	44.2
		192	20.8	2 dcm.	44.0
		250	20.5	2 dcm.	43.0
(*c*) Tetramethyl glucose	0.05 $Ba(OH)_2$	0.2	17.57	1 dcm.	74.4
		6	16.90	1 dcm.	71.6
		28	13.33	1 dcm.	56.4
		49	11.78	1 dcm.	49.9
		69	10.89	1 dcm.	46.1
		95	10.08	1 dcm.	42.7
		120	9.92	1 dcm.	42.0
		169	9.50	1 dcm.	40.2
		191	9.6	1 dcm.	40.6

TABLE IX (*Concluded*)

Sugar	Normality of alkali	Time at 35°, hrs.	Observed reading	Tube length	$[\alpha]_{\mathrm{D}}^{20^\circ}$
(*d*) Tetramethyl glucose	0.06 $Ba(OH)_2$	1.5	18.34	1 dcm.	77.7
		6	16.76	1 dcm.	71.0
		26.5	12.50	1 dcm.	52.9
		59	10.71	1 dcm.	45.3
		76	10.32	1 dcm.	43.7
		102	9.99	1 dcm.	42.3
		127	9.80	1 dcm.	41.5
		176	9.65	1 dcm.	40.9
		193	9.6	1 dcm.	41.
(*e*) Tetramethyl mannose	0.06 $Ba(OH)_2$	7	2.31	1 dcm.	9.8
		17.5	4.52	1 dcm.	19.1
		24	5.70	1 dcm.	24.1
		31	6.57	1 dcm.	27.8
		43	7.76	1 dcm.	32.9
		55	8.45	1 dcm.	35.8
		75	9.43	1 dcm.	39.9
		92	9.72	1 dcm.	41.2
		101	9.80	1 dcm.	41.5
		113	9.90	1 dcm.	41.9
		148	9.92	1 dcm.	42.0
(*f*) Equimolecular mixture of tetramethyl glucose and tetramethyl mannose	0.03 $Ba(OH)_2$	0.5	9.90	1 dcm.	41.9
		6	9.90	1 dcm.	41.9
		17.5	9.92	1 dcm.	42.0
		52.5	10.07	1 dcm.	42.6
		90	10.15	1 dcm.	43.0
		149	10.0	1 dcm.	42.3
		193	10.10	1 dcm.	42.8

From the data given in parts (*d*) and (*e*) of Table IX the rate of formation of tetramethyl mannose from tetramethyl glucose and of the reverse

TABLE X

RATE OF FORMATION OF TETRAMETHYL MANNOSE FROM TETRAMETHYL GLUCOSE AND THE REVERSE

(d)			(e)		
Interval, hours	Tetramethyl glucose, %	Tetramethyl mannose, %	Interval, hours	Tetramethyl glucose, %	Tetramethyl mannose, %
0	100.0	0	0	0	100.0
1.5	94.6	5.4	7	9.3	90.7
6	86.2	13.8	17.5	21.0	79.0
26.5	63.4	36.6	24	27.3	72.7
59	53.9	46.1	31	31.9	68.1
76	51.9	48.1	43	38.3	61.7
102	50.1	49.9	55	42.0	58.0
127	49.1	50.9	75	47.1	52.9
176	48.4	51.6	92	48.7	51.3
193	49.0	51.0	101	49.1	50.9
			113	49.6	50.4
			148	49.7	50.3

under comparable conditions may be calculated. The data are given in Table X and are plotted in Fig. 4.

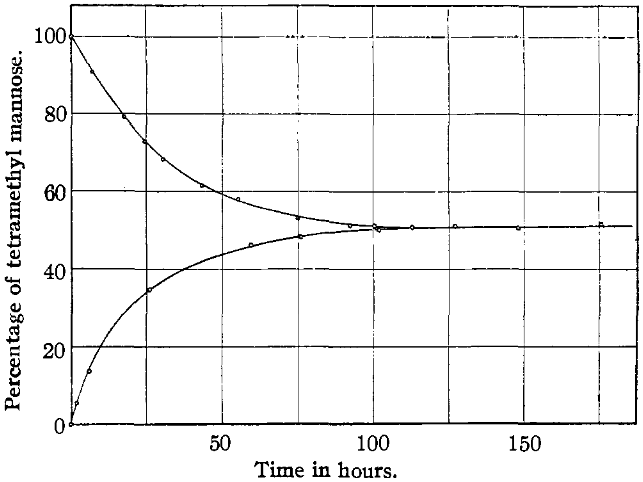

Fig. 4.—Interconversion of tetramethyl mannose and tetramethyl glucose.

Separation and Identification of the Anilides of Tetramethyl Glucose and Tetramethyl Mannose.—A weight of 35.43 g. of tetramethyl glucose was made up to 150 cc. molar solution with 0.0408 *N* barium hydroxide. This alkali was chosen in order that the metallic ion could be removed as barium sulfate. The resulting alkaline sugar solution was 0.0325 *N* alkaline. The determined density was 1.040. This solution was kept at 35° for 150 hours. The specific rotation at the end of this period was +43.4° (α = +20.51°, 2 dcm., c = molar) and the final iodine value, expressed as "apparent" aldohexose, was 138%.

The solution was then nearly "de-enolized." As some coloration occurs during this process it was deemed advisable not to carry this to completion. The molar sugar solution was diluted with sulfuric acid of such a concentration as to make the resulting solution fifth-molar sugar in 0.020 *N* sulfuric acid, allowance being made for the barium equivalent of the original solution. This diluted solution was heated rapidly in a 90° water-bath to 50° and then placed in a 50° oven for twelve hours. At the end of this period the iodine value was found to be 103% "apparent" aldohexose. The acid solution was neutralized at 40° by dropwise addition of *N*/10 barium hydroxide solution to slightly short of the neutral point and brought to neutrality by adding 0.5 g. of finely powdered barium carbonate and shaking vigorously. After standing for some time the solution was filtered and the residue washed with hot water. The filtrate was evaporated to constant weight at 25 mm. and 35°. A weight of 30.0 g. of sirup was obtained. Allowing for 2.8 g. of sugar withdrawn for samples and analysis, this represents a yield of 92%. An iodine titration of the distillate accounted for 1.5% additional if this iodine-consuming material be considered as aldohexose. Its presence in the distillate was probably due to a slight mechanical spray loss. The sirup was transferred to a 300cc. flask with absolute alcohol, removing by decantation 0.3 g. or 1% (not included in the 92% sirup figure) of brown, amorphous solid, insoluble in alcohol and ether, soluble in water and giving a test for the barium ion. A total of 95% of the original material was thus recovered.

The isolated sirup gave a specific rotation of + 42.4° (α = +2.35°, 0.943 dcm., c = 5.873) and an iodine value of 112% "apparent" aldohexose. After subtracting 2.7 g. samples and analysis, the remaining 27.3 g. of sirup was dissolved in 81 cc. of absolute alcohol, making a 30% solution, and an equal volume (8 moles) of purified, colorless aniline added. The solution was boiled gently under an air condenser for three hours. The color of the solution turned quite dark and appreciable aniline tar formation occurred which later interfered greatly with the crystallization of the anilides. On cooling of the reaction mixture and allowing this to stand, considerable crystallization occurred. The crystals were filtered and washed with aniline, 9.5 g. of crude material being obtained. On removal of the alcohol *in vacuo* a second crop of 5 g. of crystals was obtained. From the filtrate the aniline was removed *in vacuo*, a solid residue being obtained which was only slightly gummy. This residue and the two crops of crystals were recrystallized from absolute alcohol. All crops obtained in this work were dried *in vacuo* or over calcium chloride to constant weight. The mother liquors were thoroughly worked over. In this manner 27.4 g. of crystalline material was obtained in 13 crops.

The residual material, containing aniline tars, was dissolved in a small amount of alcohol and a considerable amount of tar removed by precipitation with ethyl ether and petroleum ether. After decantation from the tar, the solution was evaporated *in vacuo*, the residue dissolved in ethyl ether and a further quantity of tar removed by precipitation with petroleum ether. After removal of solvents, treatment with alcohol and refrigeration, 1.6 g. more of crystalline material was obtained in two crops. Working over the precipitated aniline tars yielded 0.1 g. of crystals.

The residual material was re-heated with aniline and alcohol and 0.6 g. more crystals obtained in two crops on working up the product with alcohol after removal of the aniline. The black, gummy material from the mother liquors of these two crops was dissolved in acetone, a relatively large volume of hot water added and steam passed through the solution. Some separation of tarry matter was thus effected. After removal of the water *in vacuo*, the residue was re-heated with aniline and alcohol and 0.5 g. more crystals so obtained. The residue was then carried through another petroleum ether tar precipitation and 0.1 g. crystals obtained. Finally, a hot extraction with 9 parts of petroleum ether and 1 part of ethyl ether and evaporation of the extract gave 0.2 g. crystals. A final, black, gummy residue of 2.5 g. remained. A total of 30.4 g. of crystalline material was obtained. All of this had been crystallized from alcohol and dried to constant weight and represents an 85% yield of anilide on the basis of the sirup used.

In order to separate and identify these anilides preliminary work was done with known pure material. It was found that the anilide of tetramethyl mannose was very appreciably more soluble in aniline than the anilide of tetramethyl glucose and a separation was effected on this basis. For identification of these compounds the following data are essential.

Tetramethyl mannose anilide has an equilibrated constant specific rotation in methyl alcohol of 8 degrees *laevo* (c = 5). This is obtained on standing for about forty hours or in a very short time on addition of 0.001% of dry hydrogen chloride to the methyl alcoholic solution. On long standing in the acid methyl alcohol, the anilide shows a slow increase in the *dextro* direction, probably due to its known ease of hydrolysis. This is at the very slow rate of about one degree in twenty-four hours. The compound crystallizes from the solvents worked with in a mixture of what is probably the two alpha and beta forms, so that melting points and initial specific rotations are unreliable.

Tetramethyl glucose anilide mutarotates in methyl alcohol to the value of fifty-nine degrees *dextro* (c = 5). Without a catalyst this value is obtained so slowly as to be unreliable. Addition of 0.001% of dry hydrogen chloride to the methyl alcohol equilibrated the solution in a short time, the final value remaining stable with the acid solution for at least twenty-two days. On one recrystallization from absolute ethyl alcohol, material that is evidently a pure form is obtained. This has a sharp melting point at 138° and shows in methyl alcohol the remarkably high specific rotation of + 226° ($c = 2$). Without a catalyst this initial value changes downward so very slowly as to be a reliable constant.

The 30.4 g. of isolated crystals were laboriously separated by means of their varying solubilities in aniline and the crystals so separated were recrystallized from absolute ethyl alcohol. The tetramethyl glucose was removed largely first, as it was the less soluble in aniline and could readily be identified by its sharp melting point. The use of aniline and repeated evaporation *in vacuo* to dryness of aniline solutions introduced aniline tars again which occasioned the loss of some crystalline material.

A weight of 14.2 g. of pure tetramethyl glucose anilide was obtained in 12 crops. The melting point of each crop was determined and varied between 137 and 138.5°. A mixed melting point of the material with a known sample of this anilide showed no depression. The initial specific rotation in methyl alcohol of the well mixed material was found to be +226° ($c = 1.998$, $\alpha = +9.04°$, 2 dcm.) which lowered to the constant value of +58.3° ($c = 1.998$, $\alpha = +2.33°$, 2 dcm.) on the addition of 0.001% of dry hydrogen chloride to the methyl alcohol.

A weight of 11.9 g. of pure tetramethyl mannose anilide was obtained in 6 crops. The well mixed material showed on equilibration a polarization value of −8° ($c = 5.000$, $\alpha = -0.38°$, 0.943 dcm.) in a methyl alcohol solution containing 0.001% of dry hydrogen chloride.

The unseparated material consisted of 0.5 g. of crystals which showed an equilibration polarization in methyl alcohol (+ 0.001% of HCl) of +38° ($c = 3.295$, $\alpha = +1.25°$, 1 dcm.). There remained 2.5 g. of gummy residual material, too dark to be polarized, and undoubtedly produced by repeated evaporation *in vacuo* of the aniline solutions. A loss in weight of 1.2 g. was suffered in separating the 30.4 g. of isolated crystals. The 26.1 g. of pure, separated crystals represents a separation yield of 86% on the basis of the isolated crystals.

Isolation and Identification of Tetramethyl Glucose and Tetramethyl Mannose.—Previous to the above separation, a rather qualitative run was made wherein the separated anilides were hydrolyzed to the sugars. These were isolated in crystalline condition and identified. The conditions used varied slightly from the previously described experiment.

Sixty cc. of molar tetramethyl glucose (14.2 g. of sugar) solution in 0.035 N (corrected for volume change) calcium hydroxide (*P*H 12.6) stood for ten days at 35°. These conditions were those used for *d*-glucose. The specific rotation (Table IX-*b*) dropped from +82 to +43° (constant) and the alkalinity was 0.008 N. The iodine value increased from 100 to 138% "apparent" aldohexose. The solution was diluted with hydrochloric acid to make a half molar solution in 3 N hydrochloric acid and stood for ten hours at 35°. The iodine value (Table VIII) rapidly decreased to 105 and then more slowly to the constant value of 100.4% of aldohexose.

The acid solution was neutralized with barium carbonate, the water removed *in vacuo*, the salt dried by distillation *in vacuo* with absolute ethyl alcohol and extracted with boiling ether. After removal of the ether, the resulting sirup, 10.9 g., gave an iodine value of 100.2% of aldohexose and a specific rotation of + 43.5° ($\alpha = +2.35°$, 1 dcm., $c = 5.462$). As the specific rotation of the solution before acid treatment was +43°, it is seen that this constant is practically unchanged by the "de-enolization."

The sirup from the ether extract was dissolved in 32 cc. of absolute ethyl alcohol to make a 30% solution, an equal volume of purified aniline (8 moles) was added and the solution refluxed for three hours. On cooling this solution to room temperature and standing for some time, 3.5 g. of crystals was filtered off and washed with aniline. A second crop, 1.6 g., of crystals was obtained on removing the alcohol *in vacuo*. From the filtrate the aniline was removed *in vacuo* and the resulting solid, 10.3 g., was leached twice with 25 cc. of aniline. One and one-half g. of residual solid was obtained. The mixed anilides, 15.4 g. or 107% of crude yield, were thus roughly separated into two very nearly equal portions of aniline insoluble, 6.6 g., and the remainder aniline soluble material.

The bulk of the aniline from the two aniline extracts, kept separate, was removed *in vacuo*. The residue was dissolved in acetone and the solution poured into water to precipitate the anilide in finely divided condition. The resulting mixture was steam distilled for three hours, the anilide passing into solution quite rapidly. This solution was evaporated *in vacuo* and the cloudy sirup so obtained freed of the slight admixture of unhydrolyzed anilide by repeatedly taking up in absolute alcohol and filtering. Four and seven-tenths g. of the resulting sirup on nucleation with tetramethyl mannose yielded 1.7 g. of plated crystals, m. p. 42–49°, $[\alpha]^{20}_{D} = +32°$ in methyl alcohol ($\alpha = + 1.11$, 1 dcm. tube, $c = 3.490$). On recrystallization from low boiling petroleum ether the following constants were obtained and are compared with those obtained by R. D. Greene[9] of this Laboratory for highly purified tetramethyl mannose.

TABLE XI

IDENTIFICATION OF TETRAMETHYL MANNOSE

Constants of crystals from mixture	Known constants
M. p. 50–51°; $(\alpha)^{20}_{D} = +27.3°$ in methyl alcohol ($\alpha = +1.00°$, 1 dcm. tube, $c = 3.668$)	M. p. 50–51°; $(\alpha)^{20}_{D} = +27.5°$ ($c = 5.00$)

The aniline insoluble material, 6.6 g., was hydrolyzed in two portions. This hydrolysis was effected by adding to a 10% acetone solution of the anilide an equal volume of 10% hydrochloric acid. This solution was heated at 50° for one hour. It was then treated with an excess of barium carbonate and the aniline removed by two hours' steam distillation in presence of the excess barium carbonate. The aqueous solution was evaporated *in vacuo*. The salt residue was dried by distillation with absolute ethyl alcohol and extracted with boiling ether. The ethereal extract was evaporated *in vacuo*. On nucleation of the resulting sirup with tetramethyl glucose, 0.7 g. of plated crystals was obtained, m. p. 81–84°, $(\alpha)_D = +85.2°$ in methyl alcohol ($\alpha = + 2.75°$, 1 dcm. tube, $c = 3.226$). On recrystallization from petroleum ether containing a trace of ethyl ether the following constants were obtained and are compared with values obtained on pure tetramethyl glucose. The 89° melting point for tetramethyl glucose is that of the pure alpha form and is obtained only on repeated recrystallization.[23] The melting point of an ordinary preparation of this sugar runs somewhat lower.

TABLE XII

IDENTIFICATION OF TETRAMETHYL GLUCOSE

Constants of crystals from mixture	Known constants
M. p. 85–86°; $[\alpha]_D = +84.2°$ in methyl alcohol; ($\alpha = +2.67°$, 1 dcm. tube, $c = 3.172$)	M. p. 89° (highest recorded; determined in this Laboratory as +83° ($c = 5$)

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[23] Purdie and Irvine, *J. Chem. Soc.*, 85, 1054 (1904).

The authors wish to acknowledge their indebtedness to Professor F. C. Whitmore for his valuable advice and suggestions.

Summary

1. The predictions of the enediol theory of sugar interconversion as applied to the methylated sugars have been experimentally verified in a study of the action of dilute alkali on tetramethyl glucose. The facts determined are as follows.

a. No keto sugars are formed by the action of dilute alkali on tetramethyl glucose.

b. By the successive action of alkali and acid on tetramethyl glucose a mixture of tetramethyl mannose and tetramethyl glucose is obtained. Evidence was found that they are the only products of the reaction and are present in equimolecular amounts. Both of these closely related, epimeric sugars were isolated in pure crystalline condition and identified. The anilides of the sugars were formed in high yield, separated and identified.

c. Strong indication has been found for the presence of the monomethyl enediol common to tetramethyl glucose and tetramethyl mannose.

2. Experimental conditions have been found for the conversion of *d*-glucose by dilute alkali into a mixture of *d*-glucose, *d*-mannose and *d*-fructose with a negligible amount of saccharinic acid formation. The composition of the equilibrated solution has been determined.

3. The normal forms of tetramethyl glucose and tetramethyl mannose, prepared by methylation of the naturally occurring sugars, are mutually interconvertible and must, therefore, possess the same oxidic ring structure unless an improbable shifting of methyl groups occurs.

4. Evidence is cited that the mechanism of enol formation is not one of selective hydration and dehydration but rather simply that of tautomeric migration of a hydrogen atom.

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