dependent on both temperature and time of exposure. Solutions of the partially dehydrated acid, when neutralized by alkali and then boiled for several hours are partially and sometimes completely restored to normal strength. In conclusion, when malic acid occurs in fruits the determination of total acid will be nearest the truth when it is made on undried samples.

AMHERST, MASSACHUSETTS

[CONTRIBUTION FROM THE UNITED COLLEGE OF ST. SALVATOR AND ST. LEONARD, UNIVERSITY OF ST. ANDREWS]

CONDENSATION OF GLUCOSE AND FRUCTOSE. SYNTHESIS OF AN ISO-SUCROSE[1]

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The investigation of which the results are now communicated has been in progress for some years and was designed to effect the synthesis of sucrose by methods based on researches carried out in this Laboratory on the constitution of mono- and disaccharides.

Prior to the study of methylated sugars initiated by Purdie and Irvine in 1901, no evidence was available regarding the particular form of glucose or of fructose present in sucrose and, in the light of later discoveries, it is not surprising that all attempts to synthesize the disaccharide by the condensation of *dextro*-rotatory glucose with *levo*-rotatory fructose resulted in failure. In 1903, however, it was shown[2] that when sucrose is methylated and the product hydrolyzed, the form of tetramethylglucose then produced is the same as that obtainable from α-methylglucoside. This result established that one half of the sucrose molecule consists of a glucose residue of normal type but, even at the time, it was evident that the fructose residue was abnormal and differed from the levorotatory form of the sugar. This was obvious from the fact that, on hydrolysis, octamethylsucrose showed no "inversion"[3] and gave a methylated fructose which, contrary to expectation, was dextrorotatory. A further important advance in the chemistry of fructose was subsequently made by Purdie and Paul,[4] who proved that two isomeric tetramethylfructoses exist, one dextro- and the other levorotatory, the reasonable conclusion being that the parent sugar would also display the same isomerism. Some years later Irvine and Robertson[5] brought forward supplementary evidence

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[1] The results described in this paper were communicated to the Institute of Chemistry (American Chemical Society) in the form of an address delivered on August 3, 1928.

[2] Purdie and Irvine, *J. Chem. Soc.*, **83**, 1036 (1903).

[3] Purdie and Irvine, *ibid.*, **87**, 1028 (1905).

[4] Purdie and Paul, *ibid.*, **91**, 289 (1907).

[5] Irvine and Robertson, *J. Chem. Soc.*, **109**, 1305 (1916).

that fructose can exist in a dextrorotatory "γ-form" and they expressed the definite opinion that sucrose must be regarded as a derivative of this variety of fructose. The above results were confirmed in the course of other researches conducted in this Laboratory on the constitution of sucrose.[6]

The synthesis of sucrose thus resolves itself into the union, through the loss of water, of equimolecular proportions of normal glucose and γ-fructose, or, alternatively, of reducing derivatives of these sugars followed by elimination of the substituting groups. In condensations of this nature the following stereochemical possibilities must be taken into account so that, in all, four isomeric gluco-fructoses may be formed.

1. α-glucose (normal type) may combine with α-fructose (γ-type)
2. α-glucose (normal type) may combine with β-fructose (γ-type)
3. β-glucose (normal type) may combine with α-fructose (γ-type)
4. β-glucose (normal type) may combine with β-fructose (γ-type)

No difficulty presents itself in obtaining a convenient derivative of glucose of the desired normal type but it is otherwise in the case of fructose, owing to the uncrystallizable nature and instability of γ-fructose derivatives. However, in the course of examining the acetylation of fructose by the method of Hudson and Brauns,[7] it was shown by Steele[8] that whereas the crystalline fructose tetra-acetate belongs to the stable type, the sirup which yields the crystalline crop consists of a mixture of partially acetylated fructoses containing γ-forms. It was hoped that this material although consisting of several compounds might be utilized as a reagent in the synthesis of sucrose [and this has subsequently been done by Pictet[9]] but in view of the importance of the problem we considered it in the highest degree desirable to use individual compounds in preference to mixtures of unknown and variable composition. The experimental obstacles encountered in realizing this program of work have been formidable. It is necessary to describe our experimental procedure in detail as, so far as can be judged, one of the methods traversed by us is precisely the same as that employed by Pictet save that we used a definite tetra-acetylfructose related to γ-fructose in place of the mixture of fructose acetates referred to above. Further, the condensations, when successful, yielded an octa-acetate isomeric and not identical with sucrose octa-acetate as the sole crystalline product containing both glucose and fructose residues. We explored, however, more than one method of coupling glucose and fructose, and for these reactions required (*a*) tetra-acetyl-γ-fructose and

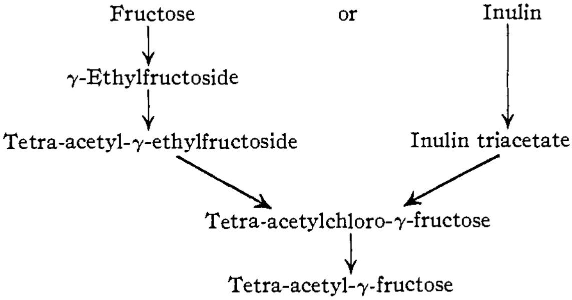
[6] Haworth and Law, *J. Chem. Soc.*, **109**, 1314 (1916).

[7] Hudson and Brauns, THIS JOURNAL, **37**, 2736 (1915).

[8] Steele, *J. Chem. Soc.*, **113**, 257 (1918).

[9] Pictet, *Helv. Chim. Acta*, **11**, 436 (1928).

(*b*) tetra-acetylchloro-γ-fructose; attention is therefore directed to the experimental part in which the preparation of these new reagents is described according to the following scheme:



At each stage the compounds formed in the above consecutive reactions were converted into tetramethylfructose in order to ascertain if the γ-type remained intact. The results were satisfactory, as the dextro-rotation and properties of the methylated ketose then isolated were in good agreement with the accepted standards for tetramethyl-γ-fructose. We have accordingly retained the expression "γ" in the nomenclature of our reagents but wish to emphasize that, as the compounds under consideration are sirups, similarities in rotation and in properties are not sound criteria in establishing identity. The history of methylated sugars shows that fundamental mistakes have been made through hasty claims of this nature and it is undesirable to urge with absolute assurance that our reagents, despite their origin and properties, belong to the same γ-fructose type as is present in sucrose.

Three distinct methods of condensation were attempted, *viz.;* A, reaction between tetra-acetylbromo- (or chloro-) glucose and tetra-acetyl-γ-fructose in the presence of a base; B, reaction between tetra-acetylglucose and tetra-acetylchloro-γ-fructose in the presence of a base; C, reaction between tetra-acetylglucose and tetra-acetyl-γ-fructose in the presence of a dehydrating agent.

It is remarkable that in Method A no condensation of the glucose and fructose residues took place. The halogen derivatives of glucose are conspicuously successful in the synthesis of β-glucosides and their failure to condense with such a reactive compound as tetra-acetyl-γ-fructose is significant. On the other hand, the use of tetra-acetylchloro-γ-fructose as the halogen-carrying reagent (Method B) was successful and the compound reacted with tetra-acetylglucose to give a crystalline octa-acetyl-*iso*-sucrose (m. p. 131–132°, $[\alpha]_D$ in chloroform + 20.3°). From this, in turn, by de-acetylation an *iso*-sucrose was isolated in the crystalline state (needles decomposing at 194°, $[\alpha]_D$ in water + 34.2°). The disaccharide, which had no action upon Fehling's solution under standard conditions but gave slight reduction on prolonged boiling, was characterized by greater instability toward acid hydrolysis than sucrose. During hydrolysis a characteristic optical inversion to give "invert sugar" took place.

Precisely similar results were obtained in the case of Method C, the same octa-acetate being formed and, in turn, the same disaccharide. Direct evidence was obtained that other gluco-fructoses were formed in both types of reaction, particularly when tetra-acetylchloro-γ-fructose was used as a reagent. In no case, however, did we obtain any crystalline derivative of a gluco-fructose other than that referred to above.

The isomerism of our synthetic disaccharide with sucrose must lie in the stereochemical form (α or β) in which the monosaccharide components are bound together, provided the oxygen ring in the fructose component is actually in the same position as in sucrose. How far this point has been safeguarded has already been discussed. In the case of our tetra-acetyl-γ-fructose, which is a sirup, both α- and β-forms are doubtless present and the same holds true for the sirupy form of tetra-acetylglucose used in the condensation, complications which are also present in Pictet's synthesis. As a general rule, however, halogen derivatives of sugars react as β-forms and this fact enables the configuration of *iso*-sucrose to be deduced with reasonable certainty. As stated, tetra-acetylchloro-γ-fructose yields the acetate of *iso*-sucrose, a result which presumably stamps the fructose residue as β. On the contrary, tetra-acetylbromoglucose, which is also β, fails to react with the acetate of γ-fructose. This might be interpreted as meaning that the α-configuration of glucose is most favorable to condensation with γ-fructose but the bulk of the evidence is opposed to this view. The reactions of tetra-acetyl-γ-fructose are not exclusively those of a monohydric alcohol and this may be the factor responsible for its failure to condense with halogen compounds. Further, when tetra-acetylchloro-γ-fructose is condensed with tetra-acetylglucose, in addition to the compound melting at 131°, a non-crystalline acetylated gluco-fructose is formed which is derived from a disaccharide showing a higher dextro-rotation than sucrose. On the basis of analogy this particular product is a derivative of α-glucoside-β-(γ)-fructose, and the compound melting at 131° must be a derivative of β-glucosido-β-(γ)-fructose. Accepting this argument, sucrose must be *either* α-glucosido-α-(γ)-fructose or β-glucosido-α-(γ)-fructose, preference being given to the latter in view of the magnitude of the rotation of sucrose and the prevalence of β-glucosides in Nature.

Since our preliminary account of the new *iso*-sucrose was published,[10] Pictet[11] has described, under the name "Saccharose D," what may possibly be the same compound.

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[10] Irvine, *J. Soc. Chem. Ind.*, **47**, 494 (1928).

[11] Pictet, *Helv. Chim. Acta*, **11**, 905 (1928).

	M. p., °C.	[α]D
Iso-sucrose octa-acetate	131–132	+20.3° (in chloroform)
"Saccharose D" octa-acetate	127	+19° (in chloroform)
Iso-sucrose	Sinters at 150° and decomposes without melting at 194°	+34.2° (in water)
"Saccharose D"	127	+19° (in water)

If the compounds are identical, Pictet's preparation must be less pure than that now described and, in any case, his allocation of the β-glucosido-β-(γ)-fructose configuration to "Saccharose D" by application of Hudson's Rule is not convincing. Calculation of the constants required to apply Hudson's Rule demands, for each form of a sugar, a knowledge of the specific rotations of pure crystalline derivatives. Such derivatives, so far as known to us, do not exist in the case of γ-fructose.

As stated, we are of the opinion that *iso*-sucrose is β-glucosido-β-(γ)-fructose but no evidence on this point is afforded by the preparation of the compound by Method C. The condensation of tetra-acetylglucose and tetra-acetyl-γ-fructose by shaking in solution with phosphoric anhydride undoubtedly proceeds smoothly and gives a mixture of acetylated disaccharides but has not, in our hands, yielded sucrose octa-acetate as a crystalline component. Consideration will show that each reagent used, in the form in which it is employed, will exist in α- and β-forms, which are capable not only of coupling but of undergoing auto-condensation. In such a reaction there is therefore the possibility that, in addition to the four isomeric glucosido-γ-fructoses, there will be formed di-glucoses and di-fructoses, the total number of octa-acetates which may be produced being ten. If the tetra-acetylfructose employed contains the stable stereoisomeride (a factor which is absent in our experiments) this number may be increased to twenty-one. It is, of course, unlikely that all the possible stereoisomerides would react equally in such condensations, but that simultaneous reactions of the above nature can and do proceed is shown by our experience that considerable quantities of *iso*-trehalose octa-acetate, together with uncrystallizable isomerides, were formed in addition to *iso*-sucrose octa-acetate.

In the course of the research tetra-acetylchloro-γ-fructose has been isolated for the first time and as this compound is obviously a valuable reagent in the study and synthesis of fructose derivatives, we desire to reserve its application and also those of the tetra-acetyl-γ-fructose described by us.

Experimental

For the purposes of this investigation the following compounds were required: (1) tetra-acetyl-γ-fructose, (2) tetra-acetylchloro-γ-fructose and (3) tetra-acetylglucose.

Preparation of Tetra-acetyl-γ-fructose from Fructose. Stage I. γ-Ethylfructoside.—The following is an account of a typical preparation. Sixty-five grams of finely-powdered fructose was dissolved in 2285 cc. of hot absolute alcohol and, after cooling, 250 cc. of alcohol containing 26 g. of anhydrous hydrogen chloride was added. The progress of the condensation was followed polarimetrically, the rotation rapidly altering to dextro and attaining in about ten minutes the maximum value $[\alpha]_D = +17°$ (approx.). The reaction was arrested as closely as possible to this maximum value, absolute alcohol, in which 18 g. of sodium had been previously dissolved, then being added. Carbon dioxide was passed through the alkaline liquor, which was thereafter evaporated under diminished pressure and the sirupy residue dried at 100° (12 mm.). This was extracted repeatedly under a reflux condenser with a mixture of ethyl acetate and alcohol until the undissolved material disintegrated into a hard red powder. The solution was then taken to dryness and the residue extracted repeatedly with dry ethyl acetate at the boiling point. On standing overnight in a corked flask a sirup was deposited from the solution and on decantation and removal of the solvent, γ-ethylfructoside remained as a sirup. It is important to note that the subsequent acetylation of the ethylfructoside should be undertaken promptly, as if the material is allowed to stand for more than twenty-four hours the acetate subsequently obtained is a hard glass and is apparently a polymeride.

Stage II. Acetylation of γ-Ethylfructoside.—This reaction was carried out in the usual manner with acetic anhydride and sodium acetate, the acetylated mixture being poured into water and nearly neutralized with calcium carbonate. Thereafter the liquid was shaken with excess of benzene, which was separated and washed with 1% sodium hydroxide until the alkaline extracts were nearly colorless. After washing with water, drying over sodium sulfate and decolorization with Norite, the benzene was removed and the residue dried at 100° (12 mm.). In this way tetra-acetyl-γ-ethylfructoside was obtained as a colorless, mobile sirup, the yield from 65 g. of fructose ranging from 33 to 47 g. As the preparation yielded a mixture of α- and β-forms, the specific rotation varied slightly in different preparations, the extremes being $[\alpha]_D$ +39.0° and +47.9° in chloroform solution ($c = 5$); n_D varied from 1.4528 to 1.4542.

Anal. Calcd. for $C_{16}H_{24}O_{10}$: C, 51.06; H, 6.38; OC_2H_5, 11.9; CH_3CO, 45.7. Found: C, 51.09, 50.75, 50.84; H, 6.36, 6.51, 6.42; OC_2H_5, 11.1, 11.8; CH_3CO, 46.3, 47.5.

For the purpose of confirming that the compound was actually a derivative of fructose, it was converted under quantitative conditions into the parent monosaccharide, a weighed quantity of the acetate being dissolved in methyl alcohol and boiled for fifteen minutes with more than the calculated amount of normal sodium hydroxide. Thereafter the alkali was neutralized and sufficient hydrochloric acid added to make the total acid concentration $N/10$. The solution was then boiled under a condenser in the presence of a small quantity of Norite and after thirty-five minutes the value $[\alpha]_D$ −84.1° was recorded, the concentration being calculated on the basis of complete conversion into fructose. In a control experiment in which pure fructose was boiled with the same concentration of aqueous acid the value $[\alpha]_D$ −85.8° was obtained in the same time. Similarly, a sample of tetra-acetyl-γ-ethylfructoside when subjected to consecutive alkaline and acid hydrolysis gave 99% of the weight of glucose phenylosazone obtained in a control experiment carried out with the equivalent amount of pure fructose.

Stage III. Hydroxylation of Tetra-acetyl-γ-ethylfructoside.—A 10% solution of tetra-acetyl-γ-ethylfructoside in acetyl chloride was saturated with dry hydrogen chloride and allowed to stand at room temperature for eighteen hours. When the reaction was complete, as indicated by the attainment of a constant rotation, the solvent was removed under diminished pressure at a temperature not exceeding 45°. The residue was dried, weighed, dissolved in benzene and an amount of silver oxide equal in weight to that of the sirup added together with a small quantity of water. The mixture was then shaken until a sample of the liquid gave no precipitate when diluted with alcohol and boiled with an alcoholic solution of silver nitrate. Water was removed by means of sodium sulfate, the solids were filtered and washed with benzene, the total filtrate being shaken with water until the extracts gave only a slight reduction with Fehling's solution. Thereafter the united aqueous extracts were shaken repeatedly with chloroform, which was separated and dried over sodium sulfate. Care had to be taken to carry out this extraction with chloroform promptly as otherwise the yields were seriously impaired. On removal of the solvent an almost colorless sirup remained which was dried at 100° (12 mm.) and consisted of tetra-acetylfructose.

Anal. Calcd. for $C_{14}H_{20}O_{10}$: C, 48.27; H, 5.74; CH_3CO, 49.4. Found: C, 48.18, 48.10; H, 5.90, 5.79; CH_3CO, 50.8; $[\alpha]_D$ in chloroform, $+31.5°$ for $c = 1.808$, and in benzene, $+38.7°$ for $c = 5.379$; $n_D = 1.4645$.

Preparation of Tetra-acetyl-γ-fructose from Inulin.—Inulin is a convenient source of tetra-acetyl-γ-fructose but the product is not so pure as when made from ethylfructoside and the process cannot be so accurately described in view of the variations shown by specimens of triacetylinulin prepared by different methods. A 10% solution of triacetylinulin in acetyl chloride was saturated with hydrogen chloride, polarimetric readings of the solution being taken at intervals. Using triacetate prepared by Pringsheim's method the specific rotation increased in four days to a value between +60° and +70°. The further procedure was exactly as described in Stage III of the preceding preparation although it proved necessary to repeat the successive treatment with benzene and with chloroform in order to obtain a colorless product. In a typical experiment 42 g. of inulin triacetate gave 32.8 g. of tetra-acetyl-γ-fructose together with 3.4 g. of a crystalline triacetyl anhydro-fructose.[12]

Anal. Calcd. for $C_{14}H_{20}O_{10}$: C, 48.27; H, 5.74; CH_3CO, 49.4. Found: C, 47.87, 47.94; H, 6.05, 5.95; CH_3CO, 49.6; $[\alpha]_D$ in chloroform, $+37.8°$ for $c = 1.838$; $n_D = 1.4662$.

Tetra-acetyl-γ-fructose shows a wide range of solubility in organic solvents and differs from the form described by Pictet in rotation and in being soluble in dry ether.

Tetra-acetyl-γ-fructose.—It is evident that by both the methods described above a form of tetra-acetylfructose is obtained isomeric with the crystalline tetra-acetylfructose of the stable type. In order to show that both preparations were derivatives of fructose, weighed amounts of each were dissolved in water containing ice and a measured excess of 2 *N* sodium hydroxide was added. The acetyl groups were completely removed when the solution was allowed to stand immersed in ice water for twenty minutes

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[12] This crystalline derivative of fructose, hitherto undescribed, was first encountered in the course of a separate research conducted in this Laboratory, the results of which will shortly be published. The compound remains behind when the benzene extract of tetra-acetylfructose is evaporated to dryness and can be obtained crystalline from the alcohol extract of the sirup.

and the excess of alkali was then estimated. Thereafter the solution was diluted to a known volume and the rotation determined. In a control experiment crystalline tetra-acetylfructose of the stable type was similarly treated, the results being:

		Acetyl content	$[\alpha]_D$
Crystalline tetra-acetylfructose		51.1	−92.4°
Tetra-acetyl-γ-fructose	From ethylfructoside	50.8	−89.8
	From inulin	49.6 (calcd. 49.4)	−89.4

As, moreover, the three compounds specified above gave yields of glucose phenylosazone comparable with that given by the equivalent weight of fructose, there can be no doubt that they are derived from this monosaccharide.

Evidence that the Dextrorotatory Tetra-acetylfructose is Derived from γ-Fructose.—Tetra-acetylfructose prepared from inulin was methylated by the action of methyl iodide and silver oxide until the reaction on Fehling's solution disappeared. The tetra-acetylmethylfructoside thus produced was then de-acetylated by the action of sodium methylate and the alkylation completed in the usual manner. In this way tetramethylmethylfructoside was formed in nearly quantitative amount and on hydrolysis by means of $N/100$ hydrochloric acid was converted into tetramethylfructose. Analysis of a distilled sample gave: C, 50.72; H, 8.59; OCH_3, 51.2. Calcd. for $C_{10}H_{20}O_6$: C, 50.84; H, 8.47; OCH_3, 52.5.

Comparison of the product with a sample of tetramethyl-γ-fructose obtained from methylated sucrose showed that the compounds displayed so close a similarity that they may be regarded as identical. The refractive indices (n_D = 1.4533) were the same and the specific rotations in water for $c = 5$ although not identical were dextro and of the same order.

	Tetramethylfructose from tetra-acetylfructose	Tetramethylfructose from octamethylsucrose
Initial $[\alpha]_D$	+26.4°	+31.8°
After 3 days	+30.1	+34.1
After 5 days	+30.1	+34.1

Taking into consideration the possibility that coincidence or similarity of optical rotations determined on liquid compounds may be fortuitous, the comparison of the two specimens of tetramethylfructose was further extended. Sodium derivatives of each were prepared by the method of Freudenberg[13] and the products showed the characteristic optical inversion, in each case the value of $[\alpha]_D$ being of the order −45°. In addition, the behavior of the two sugars when condensed with methyl alcohol was compared and likewise the rate of hydrolysis of the two specimens of tetramethylmethylfructoside thus produced. Two series of experiments were made. In one of them, 5% solutions of the two tetramethylfructoses in

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[13] Freudenberg, *Ber.*, **56**, 1119 (1923).

methyl alcohol containing 0.1% of hydrogen chloride were preserved at 15° and polarimetric records taken, initially at intervals of a few minutes and afterwards at hourly periods. The curves obtained on plotting the optical values against time were parallel (Fig. 1), although the specimen of methylated fructose prepared from sucrose showed throughout a higher dextro specific rotation of four degrees. A similar result was obtained in a second experiment in which the acid concentration was increased to 0.25% and the characteristic fall and rise of dextrorotation in the early stages of the reaction was observed in each case. In addition, the polarimetric curves representing the condensation were strictly parallel, as shown in Fig. 2.

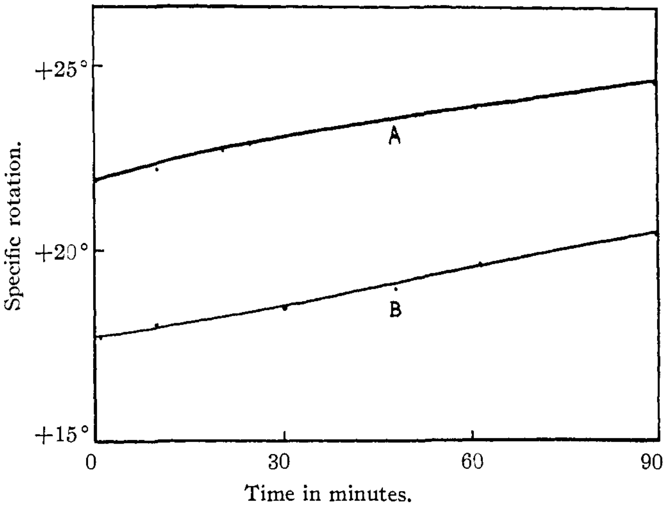

Fig. 1.—Condensation of tetramethylfructose with methyl alcohol. Acid concentration = 0.1%. T = 15°. A. Sugar obtained from sucrose. B. Sugar obtained from tetra-acetylfructose.

The specimens of tetramethylmethylfructoside produced in the reaction were isolated in the usual manner and distilled in a high vacuum, the refractive index and methoxyl content showing that the compounds were pure. Thereafter in parallel experiments the methylated fructosides were hydrolyzed by the action of $N/100$ hydrochloric acid at 85°. The polarimetric records of the hydrolysis were identical, as shown in Fig. 3, thus affording substantial evidence that the tetra-acetylfructose now under consideration is a γ-form.

Preparation of Tetra-acetylchloro-γ-fructose.—Although tetra-acetylchloro-γ-fructose may be obtained by the action of acetyl chloride on tetra-acetyl-γ-ethylfructoside, tetra-acetyl-γ-fructose is a more convenient starting-material. A 9% solution of this compound in acetyl chloride was saturated with dry hydrogen chloride and polarimetric observations were taken at short intervals. The specific rotation, which was initially +47.7°, increased rapidly, the maximum value of +56.5° being attained in about thirty minutes. At this stage the solvent was removed under diminished pressure at a temperature which must not exceed 45° and a mobile sirup remained which was dissolved in benzene. The solution was allowed to stand over potassium carbonate and on removal of the solvent a sirup remained which was essentially tetra-acetylchloro-γ-fructose. Owing to the instability of the compound no further purification was possible. On the basis of analysis the mixture contained 87% of the chloro derivative and this was confirmed by conversion of the compound by standard methods into tetra-acetyl-γ-fructose and tetra-acetyl-γ-ethylfructoside, respectively.

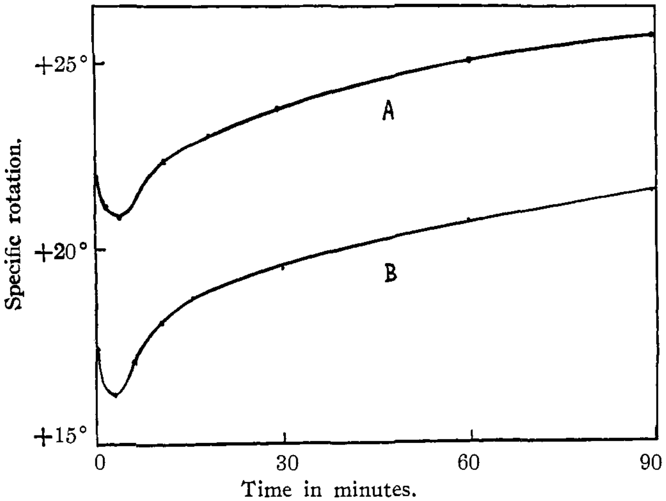

Fig. 2.—Condensation of tetramethylfructose with methyl alcohol. Acid concentration = 0.25%. *T* = 15°. A. Sugar obtained from sucrose. B. Sugar obtained from tetra-acetylfructose.

Preparation of Tetra-acetylglucose.—The method described by Fischer[14] was slightly modified. Tetra-acetylbromoglucose was dissolved in acetone and as much water added as was possible without causing precipitation. The solution was then shaken under thorough cooling with excess of silver oxide until a test portion showed that all bromine had been eliminated. The bulk of the acetone was removed under diminished pressure, excess of cold water was added and the solution extracted twice with benzene which in turn was extracted with water. The united aqueous solution and washings was shaken with chloroform, a good yield of glucose tetra-acetate being isolated from the extract in the usual way. The prod-

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[14] Fischer, *Ber.*, **42**, 2778 (1909).

uct crystallized on mixing with dry ether, the soluble sirupy variety which was used in the condensation experiments thereby passing into solution.

Condensation of Tetra-acetyl-γ-fructose with Tetra-acetylglucose.—This reaction was carried out under varying conditions, the union of the glucose and fructose residues being conducted in benzene solution by the agency of phosphoric anhydride. As the crystalline variety of tetra-acetylglucose is very sparingly soluble in benzene the non-crystalline tetra-acetyl glucose was employed. Equal weights of the glucose and fructose acetates were dissolved in benzene to give a total concentration of solids not exceeding 10%. Excess of phosphoric anhydride was added and the liquid thoroughly shaken, care being taken to ensure that the phosphoric anhydride did not settle to the bottom of the vessel. In no case did the reducing action upon Fehling's solution entirely disappear and the optimum time at which to arrest the reaction could be ascertained only by experience, but as a rule it was necessary to continue shaking for at least fifteen hours. The benzene solution was decanted and exhaustively extracted with water until the washings no longer reduced Fehling's solution. This treatment removed unchanged glucose and fructose tetraacetates, which were recovered by extracting the water with chloroform.

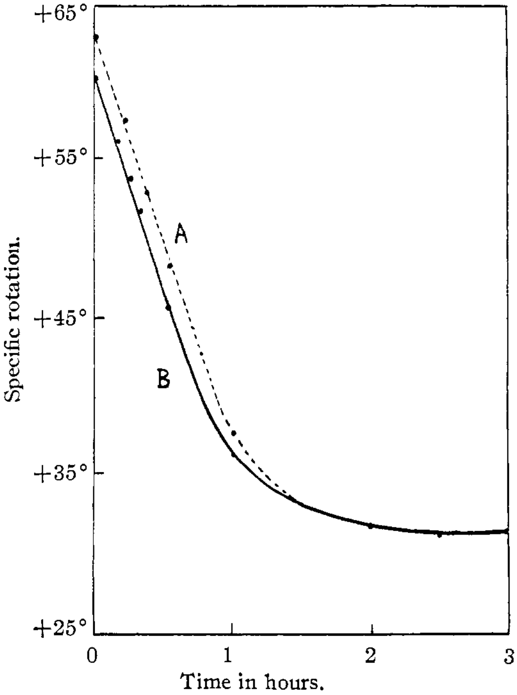

Fig. 3.—Hydrolysis of tetramethylmethylfructoside. Acid concentration = 0.36%. T = 85°. A. From sucrose. B. From tetra-acetylfructose.

The benzene was again washed, first with 1% sodium hydroxide and then with water, dried over sodium sulfate and taken to dryness under diminished pressure at a temperature not exceeding 60°. On stirring the residue with dry ether, crystals separated which were filtered, washed with ether and recrystallized from absolute alcohol. The compound, which proved to be an octa-acetyl-*iso*-sucrose, melted at 131–132°. Although this octa-acetyl-*iso*-sucrose was the only crystalline gluco-fructose derivative which was isolated, it was ascertained that *iso*-trehalose was formed simultaneously. Moreover, the yields of disaccharide derivatives were invariably small, the bulk of the materials originally employed remaining uncondensed. This is shown by the following table.

Tetra-acetylfructose and -glucose, g.	46	34.9	22.0	16.0	85.0	56.0
Acetylated monosacch. recov. unchanged, g.	34.9	22.0	16.0	4.9	56.0	..
Acetylated disaccharides, g.	7.7	4.3	4.0	7.2	10.0	9.0
Octa-acetyl-*iso*-sucrose (m. p. 131–132°), g.	(.......1.7......)			2.0	1.0	1.5

The investigation of the acetylated disaccharides other than *iso*-sucrose octa-acetate is being continued.

Condensation of Tetra-acetylchloro-γ-fructose with Tetra-acetylglucose.—To a solution of 27 g. of the chloro compound and 30 g. of sirupy tetra-acetylglucose in 135 cc. of dry chloroform were added sodium sulfate and excess of silver carbonate. The liquid was shaken until no chlorine remained in solution and after filtration the solvent was removed. On dissolving the residue in benzene and extracting repeatedly with water, reducing sugars were eliminated for the most part, while the condensation products, together with penta-acetylglucose, remained dissolved in the benzene. The solvent was evaporated, the residue dissolved in alcoholic dimethylamine and heated at 100° for three hours, reducing sugars being destroyed by the action of the base while disaccharides remained unaffected. On removal of the solvent the discolored sirup was extracted successively with ether, acetone and chloroform, most of the coloring matter thereby passing into solution. The residue was then acetylated to convert any non-reducing disaccharide into octa-acetate.

Examination of the Mixture of Disaccharide Octa-acetates.—A sample of the sirupy product, before the separation of the octa-acetate of *iso*-sucrose referred to below, was hydrolyzed under standard conditions by the successive action of alkali and acid. After removal of the acetyl groups the specific rotation was +65.9°, a value which is practically identical with that of sucrose. Moreover, on heating subsequently at 100° with $N/10$ hydrochloric acid the end-point $[\alpha]_D$ $-16.4°$ was obtained while a control experiment carried out on sucrose gave under identical conditions $[\alpha]_D$ $-19.1°$. This result proves that the disaccharide acetates under examination were convertible into "invert sugar" but the mixture, on crystallization from ether, yielded one-third of its weight of the crystalline *iso*-sucrose octa-acetate (m. p. 131–132°) already described. As the *iso*-sucrose corresponding with this acetate shows, when liberated under the same conditions, $[\alpha]_D$ +31.2°, it follows that the identity of the activity of the total product with that of sucrose must be fortuitous. Further, even if sucrose be formed in the condensation under discussion, a third isomeric gluco-fructose showing a specific rotation substantially higher than +65.9° must also be present.

Octa-acetyl-*iso*-sucrose.—This compound prepared by either of the methods described above is the only crystalline derivative of a non-reducing gluco-fructose isolated by us. It dissolves readily in hot ethyl or methyl alcohols, chloroform or acetone, is sparingly soluble in benzene or ether and is insoluble in water. When recrystallized from hot alcohol it separates in prisms.

Anal. Calcd. for $C_{28}H_{38}O_{19}$: C, 49.55; H, 5.60; CH_3CO, 50.7; mol. wt., 678. Found: C, 49.41; H, 5.61; CH_3CO, 53.0; mol. wt. in chloroform by boiling-point method, 687.

The fact that the sugar suffers slight decomposition in the presence of alkali accounts for the high acetyl value.

Solvent	Chloroform	Acetone	Benzene
c	3.762	4.072	3.918
$[\alpha]_D$	+19.9°	+20.0°	−2.0°

Iso-**sucrose.**—A 20% solution of crystalline octa-acetyl-*iso*-sucrose in absolute ethyl alcohol containing 30% of dimethylamine was heated for 150 minutes at 100° in a sealed tube. After removal of the solvent, the residue was dried at 100° (12 mm.). On dissolving this residue in the minimum amount of methyl alcohol and adding a small quantity of ethyl alcohol, a dense crop of crystals separated. These were recrystallized from the same medium, normally in the form of needles, occasionally as prisms which sintered at 152°, and decomposed sharply at 194° (uncorrected). When the sirup present in the united mother liquors was isolated, dissolved in ethyl alcohol and precipitated with ether, the total yield of de-acetylated sugar was almost quantitative.

Anal. Calcd. for $C_{12}H_{22}O_{11}$: C, 42.10; H, 6.43. Found: C, 42.09; H, 6.51.

In solubility and in crystalline form this variety of *iso*-sucrose displays a very close similarity to sucrose the difference between the two disaccharides being shown by the melting point and by the fact that this particular isomeride of sucrose is slowly decomposed on prolonged boiling with alkali and gives a small yield of glucose phenylosazone under standard conditions. The specific rotation is also markedly different from that of sucrose.

Solvent	*c*	$[\alpha]_D$
Methyl alcohol	2.130	+50.0° without mutarotation
Water	5.264	+34.2° without mutarotation

Inversion of the *Iso*-sucrose.—Although the form of *iso*-sucrose was devoid of action upon Fehling's solution for a normal period of boiling, prolonged heating occasioned a slight reduction. This indicates a greater instability toward hydrolysis than in the case of sucrose, a view which was supported by the behavior of the compound on acid hydrolysis. When heated at 100° with *N*/1000 hydrochloric acid, inversion took place, the specific rotation rapidly altering to levo and attaining the constant value

−19.2°. Under parallel conditions sucrose was hydrolyzed much more slowly, as shown from the figures quoted below:

Time, min.	*Iso*-sucrose	Sucrose
Initial	+32.8°	+66.3°
30	−16.0	+ 4.4
60	−18.2[a]	− 7.2
90	−19.2[a] (constant)	.....
100	−19.8	−13.4
135	−19.6	−15.5[a]
165	.....	−17.2[a]
195	.....	−19.2

c = 2.038; acid, $N/1000$ HCl; T = 100°.

[a] $[\alpha]_D$ recalculated for change of concentration.

The end-point is thus the same as that given by sucrose and the hydrolysis solution behaved exactly as a solution of "invert sugar" in that the optical activity vanished on heating to 80°. The yield of glucose phenylosazone from the hydrolysis liquid was also the same as that obtainable from invert sugar in equal concentration.

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Summary

1. For the purposes of the research, pure tetra-acetyl-γ-fructose and tetra-acetylchloro-γ-fructose have been prepared for the first time, and their relationship to γ-fructose established by the methylation method.

2. In attempts to synthesize sucrose from these reagents the following reactions have been carried out: (*a*) condensation of tetra-acetylchloro-γ-fructose with tetra-acetylglucose in the presence of a base, (*b*) condensation of tetra-acetyl-γ-fructose with tetraacetylglucose in the presence of a dehydrating agent.

3. In each case the only crystalline product isolated was an *iso*-sucrose octa-acetate (m. p. 131–132°; $[\alpha]_D$ +20.3° in chloroform). No sucrose octa-acetate was detected, although evidence was obtained that acetylated gluco-fructoses other than *iso*-sucrose octa-acetate and also diglucoses were formed.

4. The mixed products obtained in the above condensations gave a specific rotation practically identical with that of sucrose and are convertible quantitatively into "invert sugar." This result is, however, fortuitous.

5. *Iso*-sucrose has been isolated as needles decomposing at 194° and showing $[\alpha]_D$ +34.2° in water. The compound is hydrolyzed more readily than sucrose and is then converted into "invert sugar."

6. No union of glucose and fructose residues takes place when tetra-acetylbromoglucose is shaken in solution with tetra-acetyl-γ-fructose.

7. The collective results lead to the conclusion that sucrose is β-glucosido-α-(γ)-fructose and that the *iso*-sucrose now described is β-glucosido-β-(γ)-fructose.

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[CONTRIBUTION FROM THE BAKER LABORATORY OF CHEMISTRY AT CORNELL UNIVERSITY]

NATURAL AND SYNTHETIC RUBBER. II. REDUCTION OF ISOPRENE BY Na–NH$_3$

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The reduction of isoprene by sodium in liquid ammonia was attempted to determine: (1) whether reduction would take place in preference to polymerization and (2) the location of the added hydrogen.

Isoprene was added to sodium dissolved in liquid ammonia and a 60% yield of 2-methyl-2-butene resulted. No other volatile hydrocarbon was found. High molecular weight hydrocarbons were formed but were not investigated. It is thus shown: (1) that the predominant reaction proceeds in accordance with the equation $C_5H_8 + 2Na + 2NH_3 = C_5H_{10} + 2NaNH_2$ and (2) that hydrogen adds to isoprene in the 1,4-position, in agreement with Thiele's theory.

The hydrogen addition is similar to the bromination of isoprene at low temperature.[1] If properly conducted the latter reaction stops after 2 atoms of bromine have been added to 1 molecule of isoprene; the resulting compound, 1,4-dibromo-2-methyl-2-butene, is characterized by the inactivity of its double bond toward bromine. Similarly, 2-methyl-2-butene obtained by reduction of isoprene is not reduced to *iso*pentane by an excess of Na–NH$_3$ reagent.

Procedure.—750 cc. of liquid ammonia are placed in a one-liter round-bottomed flask (Pyrex) and 46 g. of sodium is dissolved in it. The flask is equipped with a rubber stopper carrying a separatory funnel and an abduction tube. One mole of isoprene is slowly dripped into the liquid; the stem of the separatory funnel should nearly reach the surface of the ammonia in order to prevent abduction of isoprene by the escaping ammonia vapors. When the isoprene drops strike the ammonia, a clear sound is heard which can be used to regulate the delivery. The ammonia vapors carry the reduced hydrocarbon, together with a small quantity of unchanged isoprene, through an empty safety bottle to a cylinder filled with cracked ice. Ammonia dissolves immediately, the ice melts and the water flows into a large separatory funnel; the hydrocarbon, which condenses on the ice, is carried with the water and collects as an upper

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[1] Staudinger, *Helv. Chim. Acta*, **5**, 756–767 (1922).