FURTHER ATTEMPTS TO SYNTHESIZE SUCROSE

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Researches designed to synthesize sucrose by methods consistent with the accepted constitution of the disaccharide had engaged the attention of this Laboratory for some years before Pictet and Vogel[1] announced that they had employed successfully a method which in our hands had failed to give the desired result. The only crystalline gluco-fructose isolated by us was a new isomeric disaccharide which we termed iso-sucrose and regard as β-glucose-β-(γ)-fructoside—nor did we succeed in obtaining evidence that sucrose derivatives were present in the sirupy mixture of condensation compounds from which the octa-acetate of iso-sucrose was separated.

We have not allowed these endeavors to lapse and now submit further results which amplify and confirm our previous findings and lead us to the opinion that the synthesis of sucrose is still an unattained goal. The critical point in such a synthesis lies in the necessity to obtain a tetra-substituted fructose of the γ-type containing in the reducing position a free hydroxyl group through which to couple the corresponding derivative of normal glucose. For many reasons the choice is practically limited to tetra-acetyl-γ-fructose; but this compound is a sirup presenting difficulties in purification and in the proof that during its preparation the internal oxygen linking remains in the same position as in sucrose. In order to meet these difficulties, as described in former papers,[2] we prepared our tetra-acetyl-γ-fructose by different methods and from three different sources, viz., fructose, inulin and sucrose. The products were identical, so far as can be determined with material of this description, and as they were convertible into tetramethyl-γ-fructose there can be no reasonable doubt that the fructose acetate we employed was constituted on the same model as the fructose component of sucrose. We have now prepared tetra-acetyl-γ-fructose by yet another method and in view of the importance of comparing our starting material with that used in other researches with the same objective, the constants of these different specimens of the compound are tabulated below

Source	A γ-Ethylfructoside (Irvine, Oldham and Skinner)	B Inulin	C Inulin Irvine and Stiller
Refractive index	1.4645	1.4646	1.4647
Specific rotation			
(a) in chloroform	+31.5°	+36.6°	+31.0°
(b) in benzene	+38.7°	+41.6°	+35.2°

[1] Pictet and Vogel, Helv. Chim. Acta, 11, 436 (1928), and subsequent papers.

[2] Irvine, Oldham and Skinner, THIS JOURNAL, 51, 1279 (1929); Irvine and Oldham, ibid., 51, 3609 (1929).

Source	A γ-Ethylfructoside (Irvine, Oldham and Skinner)	B Inulin	C Inulin Irvine and Stiller
Conversion into fructose			
$[\alpha]_D$ of sugar	−89.8°	−86.3°	−81.7°
Percentage yield of fructose as determined			
by controls	97.3	93.5	89.6

Process C, as now described, is the speediest and most economical method of preparation but the product possibly contains traces of glucose acetates derived from the inulin, in which case, however, the hexose impurity does not exceed 3%. It may be mentioned also that when titrated with alkali in the cold the liberated acetic acid corresponds to the presence of three in place of four acetyl groups, but this is due to the comparative stability of one acetyl group as the following evidence shows. The compound when methylated by the silver oxide reaction was converted smoothly into tetra-acetyl-γ-methylfructoside containing only one methyl group and giving the correct figures on titration with hot alkali.

The new preparation of tetra-acetyl-γ-fructose, although not entirely homogeneous, shows little variation when fractionated by dissolving in benzene and extracting repeatedly with water.

	First extract	Final extracts
Refractive index	1.4642	1.4645
$[\alpha]_D$ in chloroform	+33.9°	+31.5°
Acetyl content	38.5%	38.6%

From this evidence we are satisfied that the starting material used in the attempted synthesis now described was actually tetra-acetyl-γ-fructose of not less than 95% purity. A new method was also devised for preparing the tetra-acetylglucose required for the condensations so that we were in a position to use adequate quantities of materials and to vary the conditions of the reaction in several ways.

The method adopted to couple the glucose and fructose residues was essentially the same as before, an equimolecular mixture of the glucose and fructose tetra-acetates being dissolved in a neutral non-hydroxy solvent and shaken with phosphoric anhydride. Variations introduced included (a) the use of either benzene or chloroform as solvent; (b) alterations in the scale of the reaction from the lower limit of 8 g. up to 110 g. of the mixed acetates per experiment; (c) extension of the time of shaking from fifteen hours as a minimum to one hundred and fifteen hours as a maximum; (d) addition of the phosphoric anhydride either in one portion or in several successive installments; (e) the addition of zinc chloride in some experiments and the omission of this reagent in controls.

To economize description the conditions employed in eleven typical condensations are now synopsized in four series.

	Weight of mixed acetates, g.	Concentration, %	Time of shaking	Additions of P_2O_5
A. Condensations in Benzene without Zinc Chloride				
I	20	10	18	1
II	20	10	18	1
B. Condensations in Benzene with Zinc Chloride				
III	8	8	$22^1/_2$	2
IV	110	10	115	5
V	54	10	115	5
VI	37	10	115	5
C. Condensations in Chloroform without Zinc Chloride				
VII	52.6	8	17	2
VII (a)	85	10	15	1
D. Condensations in Chloroform with Zinc Chloride				
VIII	88.4	8	18	2
IX	12.5	8	15	1
X	12.5	8	39	3
XI	48	8	42	4

The results obtained in the above experiments are now summarized.

SERIES A AND B

	1	2	3	4	5	6
Weight of tetra-acetates used, g.	20	20	8	110	54	37
Wt. of tetra-acetates recovered unchanged, g.	12.2	11.9	2.1	40	...	16
Weight of condensation products, g.	4.1	3.9	4.3	43.3	19	13.6
Percentage yield of condensation products	20.5	19.5	53.8	39.3	35.2	36.9
Total weight of crystalline products, g.	0.8	0.8	1.1	3.5	3.9^a	0.7
Weight of iso-sucrose octa-acetate, g.	.8	.8	1.1	3.5	0.8	.7

SERIES C AND D

	7	8	9	10	11
Weight of tetra-acetates used, g.	52.6	88.4	12.5	12.5	48
Wt. of tetra-acetates recovered unchanged, g.	20.2	65.1	7.9	4.6	18
Weight of condensation products, g.	10.7	8.9	1.4	4.6	21
Percentage yield of condensation products	20.3	10.0	11.2	36.8	44
Total weight of crystalline products, g.	1.8^a	0.4^a	0.2^a	1.4^a	3.3^a
Weight of iso-sucrose octa-acetate, g.	1.2	Trace	Trace	Trace	0.9

^{a} Penta-acetylglucose formed part of the crystalline product.

The irregularity of the results renders generalization impossible but gives an index of the complexity of the reactions involved. The relative yields do not improve with large-scale working but appear to vary in proportion to the time of shaking and with the number of additions of phosphoric anhydride. As a rule, iso-sucrose octa-acetate was the sole crystalline product when the condensations were carried out in benzene solution, but when chloroform was used as solvent, particularly in the presence of zinc chloride, *the iso-sucrose octa-acetate was invariably mixed with glucose penta-acetate.* This significant result, taken in conjunction

with other evidence, gives a clue to some of the secondary reactions involved. Evidently the condensation is accompanied to some extent by the loss of acetyl groups with the consequent formation of acetic anhydride which then effects complete acetylation of the hexoses. As a further complication it may be mentioned that iso-trehalose octa-acetate was present in the crystalline product of Experiment X.

The absence of sucrose derivatives in these solid products made it necessary to focus attention on the non-crystalline condensation compounds. After removal of the unchanged glucose and fructose tetra-acetates from the sirups the acetyl content, with only one exception and that the irregular case of Experiment V, showed that from 30–45% of the material consisted of hexose penta-acetates. Further, polarimetric readings after alkaline hydrolysis followed by acid hydrolysis gave evidence that the glucose and fructose residues participated very unequally in the complex condensations.

NON-CRYSTALLINE CONDENSATION COMPOUNDS

	I	II	III	VII	VIII	IX	X	XI
COCH₃, %	59.2	56.8	60.5	60.5	59.7	52.8	60.9	...
$[\alpha]_D$ after de-acetylation	+47.6°	+39.2°	+36.4°	+74.0°	+22.2°	−13.5°	+36.6°	+66.8°
$[\alpha]_D$ after hydrolysis	+1.9°	−6.3°	−16.8°	−7.0°	−6.0°	−31.6°	−7.9°	−44.7°

In each case acid hydrolysis produces a marked fall in dextrorotation amounting generally to an optical inversion, but the irregularity of both the initial and final rotations shows clearly that, in addition to any gluco-fructoses present, some of the mixtures contained excess of di-glucoses and others excess of di-fructoses. Experiment XI is of special interest as the initial rotation is close to that of sucrose but the final value after acid hydrolysis proves that this is adventitious. This result affords a test case, as before hydrolysis all reducing components in the mixture had been removed by heating with dimethylamine and the product re-acetylated.

There remained one final possibility, *viz.*, that the results were complicated by the presence of iso-sucrose octa-acetate in each of the sirupy mixtures, thus obscuring the presence of sucrose octa-acetate and preventing its crystallization. A method was accordingly devised to decompose the less stable iso-sucrose acetate while leaving sucrose derivatives unaffected. Applying this tedious process to the product of Experiment XI as the most promising case, it was found that while the titration value remained unaffected alkaline–acid hydrolysis no longer showed an optical inversion, the change being $[\alpha]_D$ +130.8° ⟶ +35.3°. Other methods of separating any sucrose derivatives which might have been present included the formation and subsequent decomposition of calcium hydroxide compounds, but although the products showed optical inversion during acid

hydrolysis the material could not be induced to crystallize and the initial value was generally much too high in the dextro-sense for sucrose.

In one case only did we succeed in obtaining evidence which might be interpreted as indicating the presence of sucrose in small amount and it is advisable to display the sequence of reactions through which this material passed.

  I   Condensation of the glucose and fructose acetates.
 II   Elimination of unchanged glucose and fructose acetates by solution in benzene and extraction with water.
III   Removal of iso-sucrose octa-acetate from the condensation products by the action of nitric acid in chloroform solution.
 IV   De-acetylation and elimination of hexoses (present as penta-acetates) by heating with dimethylamine.
  V   Formation and decomposition of the calcium hydroxide compounds of any disaccharides present.

The product was a colorless hard glass which could not be induced to crystallize and failed to give crystalline derivatives. The analytical composition of the acetate agreed approximately with that of sucrose octaacetate and acid hydrolysis gave the inversion $[\alpha]_D + 71.6° \longrightarrow -13.9°$, figures which are close to those given by sucrose, the divergence being consistent with the presence of traces of di-glucoses. Apart from its failure to crystallize, the material was not entirely homogeneous and its formation represented no more than a 4% yield. As in work of this nature optical compensations are frequently encountered and may easily mislead, we are not inclined to attach importance to the case cited above but think it right to quote it.

Experimental

As the methods of condensing tetra-acetyl-γ-fructose with tetra-acetylglucose and of working up the products have already been detailed in former papers, the following descriptions are limited to new operations; only typical preparations are quoted.

Tetra-acetyl-γ-fructose.—Inulin acetate (20 g.) was dissolved in glacial acetic acid (200 cc.) containing 22 cc. of acetyl bromide and the solution preserved in a stoppered bottle until the specific rotation reached a maximum. This value is approximately $[\alpha]_D +80°$ and is attained in about three hours. Thereafter the liquid was transferred to a large beaker, cooled in ice and powdered ice added until the evolution of hydrogen bromide ceased. After the addition of sodium acetate to remove bromine, the solution was diluted largely with water, extracted with chloroform and the extracts dried over sodium sulfate. The chloroform was removed under reduced pressure, the residue dried at 100° and dissolved in benzene, which was extracted with water until the extracts no longer reduced Fehling's solution (15–20 extractions). The combined aqueous layers were then extracted repeatedly with chloroform which was dried and evaporated under reduced pressure. A clear sirup weighing 9.4 g. remained when dried at 100° (10 mm.); yield, 39%.

Tetra-acetylglucose.—Penta-acetylglucose (25 g.) was dissolved in 450 cc. of glacial acetic acid to which was added 50 cc. of 40% hydrogen bromide also in acetic

acid solution. In sixty-five minutes the rotation, which increased rapidly, was constant ($[\alpha]_D$ +169°). The solution was poured into 1.5 liters of cold water, 40 g. of sodium acetate added and the liquid heated on a water-bath for two hours at 55°. The product was extracted by chloroform, the extract dried and on removal of the solvent a sirup remained. This was taken up in benzene and the solution extracted with water until all reducing material had been removed. Further treatment of the aqueous extracts was as described above in the case of tetra-acetyl-γ-fructose and an average preparation gives 15–16 g. of analytically pure tetra-acetylglucose in the form of a colorless very viscous sirup; yield, 70%.

Purification of Non-crystalline Condensation Compounds. A. Removal of Unchanged Hexose Tetra-acetates.—The total products of each condensation were dissolved in benzene and extracted with water until the extracts were without action upon Fehling's solution. In this way all unchanged tetra-acetates passed into the water while hexose penta-acetates together with disaccharide octa-acetates were retained by the benzene.

B. Removal of Iso-sucrose Octa-acetate.—One method, already described and used in some cases in the present research, consists in dissolving the product from A in chloroform containing nitric acid but the following alternative is also satisfactory. A 4% solution of the sirup in glacial acetic acid containing 15% of acetyl bromide was allowed to stand until the specific rotation increased to a constant value. In the case of pure iso-sucrose octa-acetate, complete reaction takes from four to five hours, the optical activity rapidly changing from $[\alpha]_D$ +16.8 to +153.8°; a control carried out on sucrose octa-acetate under duplicate conditions showed no change in rotation for the first three and one-half hours and only a slow elevation thereafter. The reaction should not in consequence be extended beyond four hours.

The solution was poured into ice and water, a slight excess of sodium acetate added and the liquid heated at 50° until the rotation became constant, this treatment being necessary to decompose bromo-sugars. After extracting all products with chloroform, the dissolved sirup was recovered, taken up in benzene and washed repeatedly with water to remove reducing sugars. The benzene solution was finally washed with dilute caustic soda, then with water, dried and evaporated. Two objections apply to this process. Di-glucoses owing to their stability are unaffected and penta-acetylglucose remains unseparated so that the material must subsequently be passed through processes C and D.

C. Removal of Hexose Penta-acetates.—Two optional methods were employed, one being the process already described by Irvine, Oldham and Skinner[2] in which the sirup was deacetylated and the liberated hexose destroyed by heating with dimethylamine at 100° for ninety minutes: the disaccharides were thereafter recovered and re-acetylated. The alternative method consisted in dissolving the sirup in glacial acetic acid and saturating with dry hydrogen chloride. After diluting largely with water the liquid was extracted with chloroform, the extracts were dried and evaporated under reduced pressure and the resulting sirup was taken up in benzene. On extracting the solution repeatedly with water, hexose tetra-acetates passed into the aqueous layer and the purified mixture of disaccharide acetates was recovered on evaporating the benzene.

D. Separation of Disaccharides by Calcium Hydroxide.—A 10% solution of the sirup in 50% alcohol was heated on a boiling water-bath for one hour with a slight excess of calcium hydroxide prepared directly from calcium. The filtrate was decolorized with norit, taken to dryness in a high vacuum, and extracted with 90% methyl alcohol, the process of evaporation being repeated and absolute methyl alcohol used as the final extracting agent. This treatment removed uncombined disaccharide

together with calcium acetate, the mixture being re-acetylated and the disaccharide octa-acetates separated in the usual way. An amber-colored glass was obtained giving correct analytical figures and showing in a typical case the optical inversion $[\alpha]_D$ $+74.6° \longrightarrow -15.1°$ on alkaline–acid hydrolysis.

As a variation of the above process the initial sirup was de-acetylated with di-methylamine and a 10% aqueous solution boiled for one hour with calcium hydroxide. Carbon dioxide was passed through the hot filtrate for a further hour and the liberated disaccharide isolated as usual. The product was a hard clear glass which despite much endeavor could not be made to crystallize and gave the optical inversion $[\alpha]_D$ $+71.6° \longrightarrow -13.9°$ on acid hydrolysis. These figures resemble closely those given by sucrose but as other disaccharides, including di-glucoses and di-fructoses, would participate equally in all the reactions involved, we regard the resemblance as for-tuitous.

Summary

1. Additional attempts have been made to synthesize sucrose by the condensation of tetra-acetylglucose with tetra-acetyl-γ-fructose, and new methods have been devised for the preparation of these reagents.

2. Numerous variations have been introduced and the reaction has also been carried out precisely as described by Pictet and Vogel. In no case was sucrose octa-acetate obtained, the only crystalline compounds detected being iso-sucrose octa-acetate, penta-acetylglucose and iso-trehalose octa-acetate either individually or in admixture.

3. The non-crystalline products of the condensation are complex mix-tures containing acetylated diglucoses and difructoses, hexose penta-acetates, iso-sucrose octa-acetate and at least one additional gluco-fructose acetate. No evidence was obtained that sucrose octa-acetate was present in these sirups.

4. The iso-sucrose octa-acetate accumulated in the course of the re-search has been handed to Dr. C. S. Hudson, Washington, D. C., for further examination.

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