ness. The residue was extracted with hot absolute alcohol and the alcohol solution decolorized with boneblack. The bile acids were precipitated by the addition of an equal volume of ether. A separate test made on succinic acid showed that the amount which might possibly be present here would be soluble in such an alcohol-ether mixture. The filtrate from the bile acids was evaporated to dryness. The slight residue remaining was tested for succinic acid with negative results.

In the study of monobenzyl succinate a glycerol extract of the pancreas was used with the following results.

Time, hrs	.5	1	3	5	20
Alkali with ester present, cc.	86.8	87.0	87.5	87.2	86.9
Alkali of blank, cc.	10.2	10.5	11.1	10.8	10.4
Net alkali, cc.	76.6	76.5	76.4	76.4	76.5

A duplicate sample containing 1 cc. of olive oil in place of 1 g. of ester required 17.4 cc. of alkali after a 5-hour incubation; this leaves no doubt as to the activity of the enzyme preparation. The 1 g. of mono-ester would require 78.25 cc. of this alkali. Christman and Lewis[5] have previously shown that neutralization of such acid esters has little if any effect upon their hydrolysis by the pancreatic enzymes. The results would seem to indicate clearly that there was no splitting of monobenzyl succinate.

Summary

1. The lipase of the pancreas will hydrolyze dibenzyl succinate only to monobenzyl succinate.

2. Monobenzyl succinate is not hydrolyzed by this enzyme.

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[CONTRIBUTION FROM THE CARBOHYDRATE LABORATORY OF THE BUREAU OF CHEMISTRY, UNITED STATES DEPARTMENT OF AGRICULTURE]

THE PREPARATION OF FRUCTOSE[1]

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The methods described in the literature for preparing pure crystalline *d*-fructose (levulose) have not always proved satisfactory when used on a small scale to obtain this sugar for chemical and bacteriological research. Experiments in this laboratory have indicated that the causes contributing to failure in preparing levulose may be fairly assigned to the use either of impracticable methods or of methods which have been described in insufficient detail. As very uniform success has been experienced in the fractional crystallization of glucose and fructose, an attempt will be made

[1] Paper read at the 62nd meeting of the American Chemical Society, September 6–10, 1921.

in this paper to describe the method in sufficient detail to make it generally useful.[2]

Common Methods.—There are two common sources for fructose, inulin and sucrose. As inulin is a polysaccharide which on hydrolysis yields only fructose, the preparation of the sugar from this source is simpler than the procedure necessary to separate the glucose and fructose obtained by the hydrolysis of sucrose. Inulin, however, which is commonly obtained from dahlia tubers, has only a limited demand. It is, therefore, comparatively expensive and at times difficult to obtain.

For the preparation of fructose from sucrose, a method substantially as follows has usually been employed.

The sucrose is inverted with a small amount of hydrochloric or sulfuric acid and the solution of invert sugar is cooled to about 0°. Milk of lime is now added to form calcium fructosate. After purification, this salt is decomposed with carbon dioxide, sulfuric acid or oxalic acid, and the filtrate is concentrated in a vacuum to a sirup from which the fructose is crystallized by the use of alcohol. This procedure in outline has been followed in Germany by Schering, who has produced a fairly pure grade of the sugar on a commercial scale.[3]

The necessity of precipitating and handling the calcium fructosate at a low temperature, however, makes the method inconvenient and at times unreliable on a small scale.

Other methods for separating glucose and fructose have also been investigated.

Wolff[4] made use of the benzhydrazide derivative of glucose, while Adler[5] used benzidine to form diglucose benzidide. Fernbach and Schoen[6] found that an anaerobic bacillus, "gommobacter," possessed the property of attacking sucrose solutions to give almost quantitatively a gum which on hydrolysis yielded pure fructose.

Method Recommended.—The following procedure has been found very reliable in preparing fructose. It possesses the additional advantage of giving a good yield of glucose as a by-product.

A solution is made of 2000 g. of sucrose (a good quality of granulated cane sugar) in 6000 cc. of distilled water. The optical rotation of the solution is now measured, since this figure is a factor in ascertaining when the in-

[2] The experimental work on which this paper is based was completed in September, 1915. Following the author's resignation from the Bureau, the details of the method herein described were verified and the paper was prepared for publication by C. F. Walton, Jr., and John Hamilton.

[3] "Levulose, Schering" has in the past had a limited sale in this country. It has been handled by Schering and Glatz, New York, N. Y., agents for E. Schering, Berlin, Germany. When available, this commercial product is perhaps the most economical source from which to prepare the sugar on a small scale in a pure condition. Purification with basic lead acetate, the use of decolorizing carbon, and recrystallization are the only steps necessary to obtain pure fructose.

[4] Wolff, *Ber.*, **28,** 160 (1895).

[5] Adler, *ibid.*, **42,** 1742 (1909).

[6] Fernbach and Schoen, *Compt. rend.*, **155,** 84 (1912).

version is complete. To the sucrose solution at room temperature is then added a sufficient quantity of invertase to invert the sucrose completely in the desired length of time. An 18-hour period is convenient. If too long a time is required to complete the hydrolysis, fermentation may begin, the possibility of which should be reduced to a minimum. The solution is acidified with 2 cc. of glacial acetic acid, since invertase exhibits its maximum activity in a slightly acid medium.[7] The invertase may be conveniently prepared by the method described by Hudson[8] for autolyzing baker's or brewer's yeast with toluene, or it may be obtained commercially. The solution should now be allowed to stand at 20–30° until inversion is complete. The Ventzke reading for complete inversion should be almost exactly $1/3$ of the reading which has been taken before the addition of invertase, but negative instead of positive.

After the inversion has been judged complete, a few grams of active decolorizing carbon is added and the solution is filtered. The clear and colorless filtrate is then without delay concentrated in a vacuum to a sirup of about 90–95% total solids, care being taken to carry on the evaporation at as low a temperature as is possible by the use of a good water pump.[9] Since levulose is very easily destroyed by high temperatures, it is important during the entire procedure to obtain a slow evaporation at a low temperature.

The thick sirup is poured out of the flask and mixed thoroughly with 2 volumes of hot glacial acetic acid. After cooling to room temperature, it is seeded with glucose and set aside to crystallize as completely as possible at 15–20°; 3 to 4 days should be sufficient for this crystallization. The glucose is then filtered on large Büchner funnels and washed thoroughly with glacial acetic acid. The yield of glucose, dried in a vacuum oven, is 36–37.5% of the weight of sucrose taken. If very much less sugar than this is obtained, it will be difficult to get a good yield of fructose.

The filtrate, which contains the uncrystallized glucose, fructose and acetic acid, is diluted with two parts of distilled water and concentrated to a thin sirup in a vacuum at a low temperature. The water is added to lower the boiling point of the solution, so that the acetic acid may be removed at a somewhat lower temperature than would otherwise be possible. The resulting thin sirup is diluted once more with distilled water, in order that practically all the acid may be evaporated at a low temperature, and it is then concentrated to as thick a sirup as possible. The total solids of this sirup, it has been found, should approximate 90–95% as measured by the Abbé refractometer. At this point all the sirup should be contained in one 2-liter distilling flask, as it will be found easier to pour it out in this bulk

[7] Hudson and Paine, THIS JOURNAL, **32**, 774 (1910).

[8] Hudson, *ibid.*, **36**, 1566 (1914).

[9] The pump used should be capable of maintaining a steady vacuum of 710–740 mm.

than when working with a smaller quantity in a flask of the same size. The flask is drained thoroughly while the sirup is still warm. An equal volume of hot glacial acetic acid is now added and the mixture is stirred until no longer lumpy. After cooling somewhat, the mass is seeded with fructose and put away to crystallize at 15–20°. Crystallization is usually complete in 2 to 3 days.

The crystals are filtered on large Büchner funnels, washed with glacial acetic acid, and dried at a low temperature in a vacuum oven. The yield of crude sugar is 23.5–28.0% of the weight of sucrose taken.

Recrystallization.—The sugar is recrystallized from alcohol to remove all traces of mother liquor and acetic acid. For this operation, however, it is necessary to use just the right amounts of water and alcohol; otherwise fructose in the sirupy phase is thrown down by the alcohol and crystallization may then be very difficult to accomplish.

The following procedure will be found satisfactory. A solution is made of 400 g. of the crude sugar in 200 cc. of 75% ethyl alcohol on a boiling water bath, using a reflux condenser. To this solution are added 300 cc. of absolute ethyl alcohol and a small amount of active decolorizing carbon. After this mixture has been heated under the reflux condenser for a few minutes, it is filtered through a Büchner funnel. The filtrate, which is now practically colorless, is diluted with 100 cc. of absolute ethyl alcohol, stirred thoroughly, and seeded with pure *d*-fructose. The sugar is crystallized at room temperature in a desiccator, with occasional stirring as the crystallization proceeds. The yield is 75–80% of the weight of crude sugar taken.

It has been found that a second recrystallization by this same method is usually necessary to obtain pure *d*-fructose. The yield is about 80% of the weight of sugar taken. The final yield of pure sugar, from 2000 g. of sucrose, therefore, is approximately 14 to 18%. A sample of the twice recrystallized fructose, dried thoroughly in a vacuum oven, showed a constant specific rotation of $[\alpha]_D^{20}$ —92°.

Summary

Levulose was prepared by fractional crystallization from invert sugar obtained by the hydrolysis of sucrose by invertase. It was found necessary to recover by the first crystallization 36–37.5% of the weight of sucrose taken as dextrose. The yield of levulose subsequently crystallized amounted to 23.5–28% of the weight of sucrose taken. The sirups were mixed with glacial acetic acid before setting aside to crystallize. The levulose was recrystallized from alcohol.

The effect is discussed of various factors such as acidity and temperature on the crystallization of levulose.

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