[Contribution from the Carbohydrate Laboratory, Bureau of Chemistry, United States Department of Agriculture.]

THE PREPARATION OF MELIBIOSE.

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The difficulty one meets in obtaining a supply of melibiose is doubtless the explanation of the fact that this rare disaccharide is rather seldom the subject of scientific investigation. There are two reasons why the sugar is so rare: first, the only known source for it is the trisaccharide raffinose, which itself has been somewhat rare, and, second, the methods which may be found in the literature for preparing melibiose from raffinose are not satisfactory as regards the yield, in part because the details of procedure are not specified with the exactness that is necessary to enable a reader to repeat an author's preparation of a substance which crystallizes with much difficulty. Being in need recently of a supply of melibiose, we worked out the details of its preparation from raffinose, and the procedure which we recommend, after the preparation by it without difficulty of nearly a kilogram of melibiose, will presently be described.

The Source.—As mentioned, raffinose is the only known natural source for melibiose. Recently we have described in detail an inexpensive method for the preparation of raffinose from cottonseed meal[1] which yields about 4% of the weight of the meal as pure crystalline raffinose. This quantity is approximately half the raffinose that exists in the meal. Raffinose can now be prepared with certainty and at little expense and it should no longer be considered a rare sugar. For the preparation of melibiose, it is important that the raffinose be pure; it should be colorless, of correct specific rotation, without action on Fehling's solution, and should yield less than 0.1% ash.

Methods of Bau and of Loiseau for Preparing Crystalline Melibiose.—The starting point of these methods is the fermentation of raffinose by top yeast, which proceeds as follows:

$$\underset{\text{(raffinose)}}{C_{18}H_{36}O_{18}} + H_2O = \underset{\text{(melibiose)}}{C_{12}H_{22}O_{11}} + \underset{\text{(fructose)}}{C_6H_{12}O_6}$$

The yeast splits off fructose from raffinose and converts this hexose to carbon dioxide and alcohol, but the residual melibiose is not attacked by top yeast, though bottom yeast does ferment it. After the fermentation of the fructose is ended, Bau[2] decolorizes the solution with bone char, precipitates the sugar as a sirupy phase by the addition of alcohol and ether, converts the sugar to an insoluble compound with barium hydroxide, regenerates the sugar by decomposing this compound with

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[1] This Journal, 36, 2110 (1914).

[2] *Wochschr. Brau.*, 16, 397–400 (1899). See also *Chem. Ztg.*, 26, 69 (1902), and *Z. Ver. D. Zuckerind.*, 41, 481 (1904).

carbon dioxide or sulfuric acid and evaporates the filtrate, an aqueous solution of melibiose, to a thick sirup. Alcohol is added to saturation, followed by ether, and the solution is set aside to crystallize slowly. Bau does not record the yield. Bau has also prepared melibiose by hydrolyzing raffinose with weak acetic acid instead of with yeast, but the yield is not stated.

Loiseau[1] has prepared melibiose by a more direct method. After the fermentation of a 10–20% solution of raffinose with top yeast is completed, the liquid is heated, neutralized, filtered, and the filtrate evaporated to a sirup of about 30% water content, which very slowly crystallizes after being seeded with melibiose crystals. Loiseau also suggests concentrating the filtrate to 50% water content only, adding 95% alcohol until the solution is of about 70% alcoholic strength, seeding and setting aside at 15–20° for a slow crystallization. Loiseau does not record the yields of melibiose by these procedures, but in our opinion they could not have been large, because the sugar was crystallized from solutions which contained much water.

A Rapid Method for Preparing Pure Melibiose from Raffinose.—We have followed the main lines of Loiseau's method and in the following give in detail the description of a procedure by which we have uniformly obtained yields of 175–200 g. of melibiose from 500 g. of raffinose.

Five hundred grams of pure raffinose were dissolved to make approximately a 10% aqueous solution. It had a reading of +60° Ventzke in a 2 dcm. tube. From the specific rotations of raffinose (123°) and melibiose (143°), and the mass proportions of the reaction equation, it was calculated that the solution would have a reading of +47° Ventzke after fermentation with top yeast. About 10 g. of baker's yeast were added, together with a drop or two of glacial acetic acid, and about 1 g. of malt sprouts as a nitrogenous yeast food. Fermentation proceeded at room temperature. The optical rotation of the solution was observed at intervals. It rapidly fell to a value several degrees below +47°, which we attribute to the rapid hydrolysis of raffinose to melibiose and fructose by the enzyme invertase of the yeast. Since fructose is levorotatory its accumulation in the solution would cause a drop in dextrorotation of the kind observed. As the fermentation proceeded the rotation gradually increased and became stationary during some hours at 47.5°, a value slightly above the end point. This peculiarity seems to be due to the rapid removal of fructose by the yeast during the very active stage of fermentation, because if it is fermented about as rapidly as it is produced from the raffinose (and the enzymotic hydrolysis becomes slow when the raffinose concentration becomes small), the residual raffinose would cause the observed excess of dextrorotation. After 36–48

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[1] *Z. Ver. D. Zuckerind.*, 40, 1050 (1903).

hours from the start, fermentation had ceased and the rotation was found to be within 0.2° V. (46.8°) of the calculated end point. The solution was then cleared with a slight excess of basic lead acetate, filtered through an asbestos layer in a Büchner funnel, the excess of lead in the filtrate precipitated by H_2S, a quantity (30–40 g.) of active decolorizing carbon, such as "Eponit" or "Norit," added, and the solution again filtered. The excess of H_2S was removed from the filtrate with an air current and the solution boiled under reduced pressure to a sirup of approximately 20–25% water content. Alcohol (95%) was then added just below the point of the precipitation of a sirupy phase and some crystals of melibiose were stirred in. We are much indebted to Prof. J. J. Blanksma for a supply of these crystals, because the spontaneous crystallization of melibiose is very difficult to accomplish. After standing four days at a temperature near freezing, the solution had crystallized to a solid mass. The crystals were ground in a mortar with 75% alcohol at room temperature, filtered on a Büchner funnel, washed first with 75% alcohol, then with 95%, and afterwards dried in a desiccator. The melibiose was colorless and gave a correct value for its specific rotation, considering the crystals as $C_{12}H_{22}O_{11}.2H_2O$. It could be recrystallized without difficulty by dissolving to a dilute solution in water, adding decolorizing carbon, filtering, boiling under reduced pressure to a thick sirup, adding EtOH (95%) to saturation, seeding and allowing to crystallize at room temperature. In several experiments the yield has ranged between 175–200 g., as mentioned.

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A SECOND CRYSTALLINE *d*-FRUCTOSE PENTACETATE. (α-*d*-FRUCTOSE PENTACETATE.)

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Recently[1] we described the preparation and properties of a crystalline *d*-fructose pentacetate obtained by the action of acetic anhydride and sulfuric acid on *d*-fructose. We have made different attempts to convert this fructose pentacetate into the other theoretically possible isomeric form by heating with zinc chloride in acetic anhydride solution, but very little change in specific rotation was noticed and from the solution only the original substance could be recovered. On the other hand, the action of zinc chloride in acetic anhydride solution on fructose *tetracetate* caused a considerable change in specific rotation and it seemed that an isomeric fructose tetracetate might be obtained. However, the crystalline sub-

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[1] THIS JOURNAL, 37, 1283 (1915).