

In carrying out the test the ammoniacal filtrate is taken from the bismuth separation, the excess of ammonia is removed either by boiling or by careful neutralization with acid. If sufficient ammonium salts have not been added in the course of the analysis, 3 or 4 cc. of a 10% solution of ammonium chloride is added at this time. The solution is then treated with an equal volume of saturated sodium bicarbonate solution. The presence of cadmium is indicated by a turbid, white precipitate which is easily noted in contrast to the clear blue of the copper solution.

The test is sensitive to about 0.1 mg. per cc., which can be detected without difficulty in the presence of five times that amount of copper. Failure to remove mercury or lead before the bismuth separation does not interfere with the test.

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## THE OCCURRENCE OF GENTIOBIOSE IN THE PRODUCTS OF THE COMMERCIAL HYDROLYSIS OF CORN STARCH<sup>2</sup>

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### Introduction

In the manufacture of crystalline *d*-glucose by the hydrolysis of corn starch with weak hydrochloric acid, a final mother liquor is obtained that is of great concern to the industry because of its quantity and its undesirable influence upon the rate of crystallization and yield of the sugar. This mother liquor, commercially known as "hydrol," consists approximately of 70% of fermentable and 30% of unfermentable material on the basis of dry substance. The fermentable part of "hydrol" is chiefly *d*-glucose. This conclusion follows from a comparison of the weight of carbon dioxide developed during fermentation and the accompanying loss in reducing sugar. The unfermentable part resembles closely in physical and chemical properties the saccharine material that is known in sugar chemistry as "isomaltose." This name was first used by Emil Fischer<sup>4</sup>

<sup>1</sup> Published by permission of the Director of the Bureau of Standards, United States Department of Commerce.

<sup>2</sup> An abstract of this paper was presented before the Sugar Division of the American Chemical Society at the meeting held in Los Angeles, California, August, 1925.

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<sup>4</sup> Fischer, *Ber.*, 23, 3687 (1890).

to signify a synthetic, sirupy disaccharide that he obtained by treating *d*-glucose with concentrated hydrochloric acid. He described it as an unfermentable sirup which yielded a crystalline phenylosazone of the composition  $C_{24}H_{32}N_4O_9$ . This in turn was converted to an osone that on hydrolyzing gave *d*-glucose and *d*-glucosone, which are in like manner obtained by hydrolyzing maltose osone. Fischer's isomaltose osazone, which he states could not be satisfactorily purified, melted at  $158^\circ$  and showed  $[\alpha]_D = +7$  in alcohol. Ost<sup>5</sup> prepared "isomaltose" by treating *d*-glucose, or maltose, with sulfuric acid of about 30% strength. He described it as an unfermentable, non-crystallizing disaccharide which showed  $[\alpha]_D = +70$ , possessed a reducing power about 66% of that of maltose, and formed a phenylosazone melting at  $130$ – $145^\circ$ , and showing  $[\alpha]_D = -15$  to  $-20$ . O. v. Friedrichs<sup>6</sup> treated *d*-glucose with fuming hydrochloric acid and obtained a non-fermentable sirup which he considered to be the same as Fischer's "isomaltose" because it showed  $[\alpha]_D = +73$  and yielded a phenylosazone melting at  $146^\circ$  and corresponding in analysis to a disaccharide derivative.

The purified, unfermentable part of hydrol was found in the present investigation to show  $[\alpha]_D^{30} = +70$ , and its reducing power was 66% of that of maltose or 40% of that of dextrose. The phenylosazone prepared from it corresponded in analysis to that of a disaccharide, melted at  $150$ – $152^\circ$  and showed  $[\alpha]_D^{25} = -20$  in methyl alcohol. Evidently the unfermentable part of hydrol resembles closely the products that have been described as "isomaltose."

Criticism may properly be made, however, of applying the name "isomaltose," which should presumably indicate a definite sugar, to a sirupy product that may consist of a mixture of various carbohydrates. Indeed, the present investigation shows that isomaltose is such a mixture.

The fact that the acetyl derivatives of many of the sugars are soluble in a variety of solvents and frequently crystallize well, led to the attempt to separate and identify the unfermentable components of hydrol through the method of acetylation. The results of the experiments are, so far, the isolation from the acetylated unfermentable part of hydrol of a crystalline acetyl derivative which was readily identified as  $\beta$ -octa-acetyl gentiobiose. After recrystallization from absolute methyl alcohol the substance melted at  $195$ – $196^\circ$ , and showed the same melting point when mixed with pure  $\beta$ -octa-acetyl gentiobiose; its specific rotation in chloroform was found to be  $[\alpha]_D^{26} = -5.61$ .<sup>7</sup> The yield, based on the total amount of dry substance and mother liquor used, corresponded to about

<sup>5</sup> Ost, *Chem.-Ztg.*, 20, 762 (1896).

<sup>6</sup> v. Friedrichs, *Arkiv Kemi, Min. Geol.*, 5, 4 (1913); *Chem. Zentr.*, 85, 763 (1914).

<sup>7</sup> Zemplén, *Ber.*, 48, 235 (1915). Hudson and Johnson, *THIS JOURNAL*, 39, 1272 (1917).

5.7% of free gentiobiose. Based on the amount of unfermentable material used, the yield was about 18.3% of free gentiobiose. Since  $\beta$ -octa-acetyl gentiobiose is very sparingly soluble in absolute methyl alcohol and readily crystallizes from this solvent, it seems safe to assume that the yield mentioned represents the main part of gentiobiose present in the acetylated mixture, and from this it follows that gentiobiose represents a relatively small portion of the unfermentable part of hydrol. This is also clearly indicated by the specific rotation of the unfermentable part of hydrol ( $[\alpha]_D = +70$ ), since the  $[\alpha]_D$  of gentiobiose is  $+9.8$ .

### Experimental Part

**The Preparation of  $\beta$ -Gentiobiose Octa-acetate from Hydrol.**—Four hundred g. of *d*-glucose mother liquors ("hydrol") containing about 75% of solids was dissolved in 2600 cc. of water and to the solution 25 g. of baker's compressed yeast was added. The addition of mineral salts, etc., to stimulate the activity of the yeast was found to be unnecessary. After allowing the liquid to ferment at 28–30° for one week, it was boiled for 15 minutes, cooled to room temperature, neutralized with a cold saturated solution of barium hydroxide, again brought to a boil, and filtered hot. The filter cake was washed with hot water and the filtrate and wash water were then combined and evaporated in a vacuum to about 1500 cc. This solution was then treated with decolorizing carbon, filtered and evaporated in a vacuum until the weight of the sirup was about 200 g. This was then refluxed for three hours with 1500 cc. of absolute methyl alcohol, kept at room temperature overnight, and the alcoholic solution poured off. The thick, sirupy residue was refluxed twice again with 500 cc. of absolute methyl alcohol. The alcoholic solutions were combined and ether was added until no more precipitate formed. The ether-alcohol solution was poured off after it had become clear on standing and the amorphous residue was dried in a vacuum desiccator over lumps of caustic soda and paraffin at ordinary temperature. The yield was 93 g. of dried residue, or 31% of the total solids of the "hydrol." This residue in aqueous solution showed the specific rotation  $[\alpha]_D^{30} = +70.4$ . Twenty g. of it was acetylated with 240 g. of acetic anhydride (99.2%) and 120 g. of pyridine at 55–60° with occasional stirring until solution was complete. The solution was kept at room temperature overnight and then poured into 4 liters of ice water. The insoluble portion solidified after several hours to a brittle mass which was ground, filtered, washed with cold water and dried in a vacuum desiccator over caustic soda and paraffin; yield of acetyl derivative, 27.5 g. This material was dissolved in 100 cc. of ether and the solution was washed with water until neutral, then with 3% sodium bicarbonate solution and again with water to neutrality. The ethereal solution soon deposited crystals (in one case even before washing was completed) in the form of long needles; yield, 2 g. After recrystallization from absolute methyl alcohol they melted at 195–196°, softened at 192–193° and showed  $[\alpha]_D^{25} = -5.61$  in chloroform. These properties agree closely with those of pure  $\beta$ -gentiobiose octa-acetate. The ethereal filtrate was evaporated to dryness, the residue was dissolved in a little methyl alcohol, and the solution was seeded with some of the crystals previously obtained. In time, 5.26 g. of pure  $\beta$ -gentiobiose octa-acetate crystallized, making the total yield 7.26 g. This corresponds to about 17 g. of gentiobiose per 300 g. of dry solids of hydrol, or about 5.7%.

**Preparation of a Phenylsazone Similar to Phenyl Isomaltosazone.**—Ten g. of the unfermentable residue (soluble in methyl alcohol) was dissolved in 50 cc. of water and to the solution 10 g. of phenylhydrazine and 7 g. of 50% acetic acid were added; the mixture was heated on the water-bath for one and one-half hours. Upon cooling and

standing, yellow needles separated which were recrystallized from 70% methyl alcohol; m. p., 150–152°;  $[\alpha]_D^{25} = -20.24$  in methyl alcohol. They were dried in a vacuum desiccator over sulfuric acid for analysis.

*Anal.* Calcd. for  $C_{24}H_{32}N_4O_8 \cdot H_2O$ : C, 53.50; H, 6.37; N, 10.42. Found: C, 53.29, 53.05; H, 6.46, 6.38; N, 10.64, 10.50.

The author is indebted to Dr. C. S. Hudson for helpful suggestions.

### Summary

Gentiobiose has been identified through the isolation of its  $\beta$ -octa-acetate in pure, crystalline form, as one of the constituents of the mother liquor ("hydrol") obtained in the commercial manufacture of crystalline *d*-glucose.

It has been shown, through a comparison of physical and chemical properties, that the unfermentable part of hydrol, while closely resembling isomaltose, contains only a comparatively small amount of gentiobiose, and criticism, therefore, is made of applying the name "isomaltose," which should presumably indicate a definite sugar, to a product that apparently consists of a mixture of carbohydrates.

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

## STUDIES IN VAPOR PRESSURE. II THE MONONITROTOLUENES

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Many of the earlier determinations of the vapor pressures of the more important organic compounds that enter into the manufacture of dyes are unreliable owing to the lack of refined instruments for making observations and to the impurity of the materials measured. As a result of these conditions, the National Research Council has suggested that the vapor pressures and boiling points of these compounds be determined by the more accurate methods now available. This paper gives the results of a study of the vapor pressures of the three isomeric nitrotoluenes, and their latent heats of vaporization and entropies have also been derived. The method used to measure the vapor pressures and the means employed to calculate their vapor-pressure equations, latent heats of vaporization, and entropies are given in detail in a recent communication.<sup>1</sup>

### Materials

The nitrotoluenes used in this investigation, which were the purest procurable, were further purified as follows.

The *o*-nitrotoluene was distilled, the first and last sixth of the distillate being dis-

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<sup>1</sup> Berliner and May, *THIS JOURNAL*, **47**, 2350 (1925).