

above what seemed to be the true $\Pi n:PH$ curve. The solutions, at the mid-point of reduction, were dark brown in color, that is, deeper in color than the quinone solution itself.

While no satisfactory account of these peculiarities suggests itself, it is of interest that Sullivan, Cohen and Clark¹⁵ encountered entirely analogous discrepancies in this same region of alkalinity in their investigation of the electrode potentials of indigo sulfonates. While these authors considered the effect specific to borate buffers, though indeed certain divergences with alkaline phosphate buffers were recorded, no differentiation between borate and phosphate buffers was apparent in the present case. It seems likely that some phenomenon not yet understood is in operation at the particular alkalinity at which few but borate buffers are available. Thus glycine buffers are out of the question because the sulfonated quinones react with glycine.

Only those results about which there is no cause for uncertainty have been included in the table. Fortunately, these results furnish the comparison desired.

In conclusion I wish to express my gratitude to Professor William Henry Perkin, Jr., for his many kindnesses during a portion of this work.

Summary

Sodium 6,7-indazole-4-sulfonate has been prepared and characterized and compared with potassium β -naphthoquinone-4-sulfonate. The normal reduction potentials of the two substances at 25° are 0.620 v. and 0.630 v., respectively, which indicates a close relationship between the pyrazole and the benzene systems.

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THE IDENTITY OF ISOMALTOSE WITH GENTIOBIOSE

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Introduction

The literature on isomaltose is quite extensive³ yet the constitution of this sugar is still the subject of controversy and uncertainty, and some

¹⁵ Sullivan, Cohen and Clark, *Pub. Health Repts.*, **38**, 1669 (1923).

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³ E. O. v. Lippmann, "Die Chemie der Zuckerarten," Vieweg, Braunschweig, 1904, Vol. II, pp. 1504-1520. E. Abderhalden, "Biochemisches Handlexikon," Springer, Berlin, 1923, Vol. X, pp. 608-609.

authorities go even so far as to question the existence of the disaccharide. This is due to the fact that the investigators of isomaltose have not been able to agree on the physical and chemical properties of this sugar.

The characteristic properties of isomaltose on which all investigators agree are, first, its inability to ferment with brewer's yeast and, second, its ability to form a phenylosazone. But in so far as the specific rotation of the sugar, or the melting point and specific rotation of its phenylosazone are concerned, different investigators have different claims. While this does not necessarily indicate that the isomaltoses of different investigators are different sugars, it at least indicates that they are products of different degrees of purity, depending upon the particular method of preparation.

Isomaltose has never been obtained in its pure, crystalline form, and for determining its specific rotation a product sometimes more, sometimes less crude, but always crude, has been used. Under such circumstances one should hardly expect to obtain a correct value for $[\alpha]_D$. The osazone reaction, on the other hand, is not always a sure method for identifying a sugar, first, because the mechanism of this reaction is complicated and easily accompanied by side reactions; second, because different sugars often form identical osazones and, third, because osazones are often difficult to purify. The melting point of an osazone, its optical activity and the possibility of purifying it thus depend upon the nature and the amount of the impurities present in the reaction mixture during its formation.

It has been found by the writer⁴ that the unfermentable part of the product which is obtained by hydrolyzing corn starch with weak hydrochloric acid for the purposes of manufacturing *d*-glucose, while closely resembling in its physical and chemical properties isomaltose, contains gentiobiose. It, therefore, appeared probable that the so-called isomaltose osazone which Fischer first isolated in low yield⁵ from so-called isomaltose, which was made from *d*-glucose by the condensing action of concd. hydrochloric acid, might be the osazone of gentiobiose, possibly in impure form. Gentiobiose was unknown at the time of Fischer's work upon isomaltose, it having been discovered by Bourquelot and Herissey ten years later.⁶ Fischer states that he was unable to fully purify his isomaltose osazone.

Using Fischer's method for preparing isomaltose by treating *d*-glucose with concd. hydrochloric acid,⁷ and Friedrich's⁸ method for working up the reaction product, a sirup was obtained from which upon acetylation a crystalline substance was isolated and identified as β -octa-acetyl gentiobiose.

⁴ Publication of the research is now in progress.

⁵ Fischer, *Ber.*, **28**, 3024 (1896).

⁶ Bourquelot and Herissey, *Compt. rend.*, **132**, 571 (1901).

⁷ Fischer, *Ber.*, **23**, 3687 (1890).

⁸ Friedrich, *Arkiv Kemi, Mineral Geol.*, **5**, No. 4 (1913); *Chem. Centr.*, **85** (I), 763 (1924).

Reference may be made here to the work of Zemplén⁹ who sought some years ago to isolate gentiobiose octa-acetate by the acetylation of crude isomaltose sirups, but was unable to obtain the substance and therefore concluded that gentiobiose and isomaltose are different sugars. Apparently the details of procedure are very important because by the same general course, as described specifically below, there has been obtained without difficulty in the present work a yield of about 2 g. of crystalline gentiobiose octa-acetate per 100 g. of original glucose.

Upon saponifying β -octa-acetyl gentiobiose and treating the product with phenylhydrazine in the usual manner, a crude phenylosazone was obtained which had the same melting point and crystalline appearance as Fischer's isomaltose phenylosazone. It showed $[\alpha]_D^{20} = -42.9$ (final) in alcohol and melted at $156-8^\circ$, while Fischer's osazone showed $[\alpha]_D = +7$ in alcohol and melted at 158° . The discrepancy in rotation may be due to the impurities in Fischer's osazone which, as stated above; he was unable to remove. His actual reading was only 0.2° , and therefore the measurement is subject to much uncertainty. After recrystallization from hydrous ethyl acetate in the present investigation the osazone melted at $179-181^\circ$ and showed $[\alpha]_D^{22} = -70.8$ immediately after dissolving in a mixture of four volumes of pyridine and six volumes of 95% alcohol, and $[\alpha]_D^{22} = -48.2$ after 18 hours' standing. Zemplén¹⁰ prepared gentiobiose phenylosazone from the fermented product obtained by the action of emulsin upon *d*-glucose and obtained a product which melted at $160-170^\circ$ and showed $[\alpha]_D^{20} = -74.8$ in a mixture of four volumes of pyridine and six volumes of alcohol. This agrees closely with the observations in the present work if Zemplén's reading was made upon a freshly prepared solution. Apparently such was the case because he does not mention observing the mutarotation. The higher melting point obtained in the present investigation indicates a greater purity of the osazone. After that portion of acetylated isomaltose from which gentiobiose octa-acetate has been removed by crystallization had been saponified, no osazone could be obtained from it.

These experiments seem to justify the conclusion that Fischer's isomaltose is identical with gentiobiose, the definition of isomaltose being that it is the sugar which is the parent substance of Fischer's isomaltose phenylosazone.

Experimental Part

The Preparation of Isomaltose.^{7,8}—A solution of 100 g. of pure *d*-glucose dissolved at room temperature in 400 g. of hydrochloric acid, *d.* 1.19 (37%), was allowed to stand at $8-10^\circ$ for 24 hours. The reaction mixture, after 500 g. of ice had been added to it, was neutralized with a suspension of neutral lead carbonate in water. After the separated lead chloride had been filtered off and washed with a small amount of ice water,

⁹ Zemplén, *Ber.*, **48**, 234 (1915).

¹⁰ Ref. 9, p. 236.

the combined filtrate and wash-water was treated with 15 g. of freshly precipitated silver carbonate, again filtered and then saturated with hydrogen sulfide. After filtering off the sulfides and passing air through the boiling filtrate in order to expel the excess of hydrogen sulfide, another portion of silver carbonate was added. Upon removing the separated silver chloride the filtrate, still hot, was treated with several drops of ammonium sulfide solution, filtered and boiled for five minutes. The final filtrate was made up to a volume of 3000 cc. and fermented with 15 g. of compressed baker's yeast at 32–34° during 66 hours. The fermented liquor was decolorized with active carbon and evaporated in a vacuum to about 500 cc. It was then neutralized with powdered calcium carbonate, filtered, and evaporated in a vacuum as dry as possible. The residue, weighing 25 g., was extracted with 250 cc. of hot absolute methyl alcohol, and the extract poured into 350 cc. of ether. The precipitate was filtered off and dried in a vacuum over lumps of sodium hydroxide and paraffin at ordinary temperature. The yield was 15 g. of dried residue.

Acetylation of Isomaltose and Separation of β -Gentiobiose Octa-acetate from the Acetylation Product.—Fifteen g. of dried isomaltose residue was heated on the water-bath with a mixture of 65 g. of pyridine and 120 g. of acetic anhydride (90–95%) during two hours and occasional shaking. The resulting solution was kept at room temperature overnight and then poured into 4 liters of cold water. The insoluble portion solidified after several hours to a brittle mass which was ground, filtered and washed with cold water. The filter cake, still wet, was dissolved in 100 cc. of ether and the solution washed with water until neutral, then with 1% sodium bicarbonate solution and then again with water to neutrality. The ethereal solution soon deposited crystals in the form of long needles; yield, 1.2 g. After recrystallization from absolute methyl alcohol they softened at 192–193°, melted at 195–196° and showed $[\alpha]_D^{24} = -4.4$ in chloroform. These properties agree closely with those of β -gentiobiose octa-acetate. Upon the addition of petroleum ether to the ethereal filtrate about 0.8 g. more of β -gentiobiose octa-acetate crystallized, making the total yield about 2 g. This corresponds to about 1 g. of gentiobiose per 100 g. of *d*-glucose, or about 1%.

Preparation of Gentiobiose-Phenylosazone from β -Gentiobiose Octa-acetate.—A suspension of 7.2 g. of β -gentiobiose octa-acetate in 50 cc. of absolute methyl alcohol was saponified by adding a solution of 0.36 g. of metallic sodium in 10 cc. of absolute methyl alcohol and allowing the mixture to stand for 15 minutes during occasional shaking; 25 cc. of water, 4 g. of glacial acetic acid (99.9%) and 6 g. of phenylhydrazine were then added and the mixture was heated on the water-bath for one and a half hours. On cooling, the solution deposited crystals of the osazone which were filtered off and washed with cold water; yield, 2.1 g., or 38%; m. p., 156–158° after drying in a vacuum desiccator over sulfuric acid at room temperature to constant weight. A solution of 0.6005 g. of osazone in 95% ethyl alcohol to a volume of 50 cc. showed $[\alpha]_D^{20} = -42.9$. On the addition of water to the filtrate 0.8 g. more of the osazone separated, making the total yield 2.9 g., or 52.5%. After recrystallization from hydrous ethyl acetate the osazone, consisting of long needles, melted at 179–181°, and 0.5295 g. dissolved to a volume of 25 cc. in a mixture of four volumes of pyridine and six volumes of 95% alcohol showed $[\alpha]_D^{22} = -70.8$ immediately upon dissolving, and $[\alpha]_D^{22} = -48.2$ after 18 hours.

The author is indebted to Dr. C. S. Hudson and Dr. A. Kunz for valuable suggestions.

Summary

The crude sirup, representing the unfermentable portion of the product obtained by the action of concd. hydrochloric acid solution upon *d*-glucose

as described by Emil Fischer and by Friedrich, has been acetylated and crystalline β -gentiobiose octa-acetate has been obtained in a yield corresponding to about 1 g. of gentiobiose per 100 g. of *d*-glucose. Evidence is presented that the disaccharide osazone which Fischer obtained in low yield from such a sirup, and named isomaltose osazone, is gentiobiose osazone.

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NOTE

p-Nitrobenzoyl Esters of β -Phenylethyl, γ -Phenylpropyl, and δ -Phenylbutyl Alcohols.—In the preparation of normal ω -phenyl-alkyl chlorides containing from one to seven carbon atoms in the chain, the corresponding alcohols were obtained as intermediates.¹ In order to characterize these alcohols definitely it was desirable to convert them into solid derivatives for the purpose of analysis.

It had been found that the phenylurethan derivatives were not suitable for this purpose, inasmuch as these substances all had relatively low melting points which decreased with increasing length of the carbon chain. The decrease in melting point is not regular but gives indication of alternation. ϵ -Phenylamyl alcohol did not yield a solid phenylurethan even when cooled to low temperatures. Table I gives the melting points of the phenylurethan derivatives.

TABLE I
MELTING POINTS OF PHENYLURETHAN DERIVATIVES

Bz = benzyl; Ph = phenyl; Et = ethyl; Pr = propyl; Bu = butyl; Am = amyl; Hx = hexyl; Hp = heptyl.

Phenylurethan of	Phenol ^a	Bz alc. ^b	β -PhEt alc. ^{c,d}	γ -PhPr alc. ^e
M. p., °C.	125.5	78	79-80	47-48
Phenylurethan of	δ -PhBu alc. ^e	ϵ -PhAm alc. ^e	ζ -PhHx alc.	η -PhHp alc.
M. p., °C.	51-52	uncrystallizable	?	?

^a A. W. Hoffmann, *Ber.*, **4**, 249 (1871). Eckenroth, *Ber.*, **18**, 517 (1885). Leuckhart, *Ber.*, **18**, 875 (1885). Cazeneuve and Morel, *Bull. soc. chim.*, [3] **19**, 696 (1898). A. Morel, *ibid.*, **21**, 827 (1899).

^b Soden and Rojahn, *Ber.*, **34**, 2809 (1901).

^c H. Walbaum, *Ber.*, **33**, 2300 (1900).

^d Soden and Rojahn, *Ber.*, **33**, 3065 (1900).

^e von Braun, *Ber.*, **44**, 2872 (1912).

A few of the *p*-nitrobenzoyl esters of this series of alcohols were prepared with the hope that perhaps they would yield solids of higher melting point than the phenylurethans. It was found, however, that in case of these derivatives also the melting points decreased rapidly, but without alternation, and that even the ester of δ -phenylbutyl alcohol had a melting point

¹ Conant and Kirner, *THIS JOURNAL*, **46**, 240 (1924).