

[JOINT CONTRIBUTION FROM THE DEPARTMENTS OF CHEMISTRY AND PHYSIOLOGICAL CHEMISTRY, YALE UNIVERSITY]

STUDIES ON ACETOL. I. A NEW TEST FOR CARBOHYDRATES

BY OSKAR BAUDISCH AND HARRY J. DEUEL

Received April 26, 1922

In the previous paper of Deuel and Baudisch,¹ it was shown that thymine can be oxidized by the use of ferrous hydroxide-peroxide to urea, acetol and pyruvic acid. Acetol is also formed, as Baudisch² has shown, by the decomposition of glucose with alkali. Even an alkali as weak as sodium hydrogen carbonate gives, on boiling with a water solution of glucose, the characteristic reaction for acetol with *o*-amino-benzaldehyde.

Formerly it was believed that the strongly reducing, volatile substance formed when a sugar solution is heated with alkali, is methyl glyoxal because it gives the same osazone as acetol. Since the new reaction of Baudisch was developed, it has become possible to distinguish between methyl glyoxal and acetol, since methyl glyoxal does not react with *o*-amino-benzaldehyde or at least does not give the fluorescent 3-oxy-quinaldine. This reaction is discussed at greater length below.

G. Pinkus,³ who made the osazone of methyl glyoxal by boiling glucose in caustic soda solution in the presence of phenylhydrazine, believed that acetol is formed first as an intermediate product and is converted into methyl glyoxal by phenylhydrazine. Nef,⁴ A. Wohl,⁵ and C. Neuberg⁶ believe that we must consider methyl glyoxal to be the mother substance. Probably by decomposing sugar in alkaline solution both substances are formed. It is quite possible that acetol and pyruvic acid may be formed by the influence of alkali as a result of a Cannizzaro reaction. Methyl glyoxal, which had been made according to the description of Denigé by the oxidation of glycerol with bromine, gave on distillation with sodium hydrogen carbonate a very small amount of acetol. This experimental result seems to indicate that on the decomposition of sugar in alkaline solution acetol may be formed as a primary product, since approximately 1 g. of methyl glyoxal gives only a faint acetol test, while 5 mg. of glucose readily gives a decided test for it. We propose to study the Cannizzaro rearrangement of methyl glyoxal thoroughly as soon as sufficient chemically pure material is available. For the present, we are interested in the fact

¹ Deuel and Baudisch, *THIS JOURNAL*, **44**, 1581 (1922).

² Baudisch, *Biochem. Z.*, **89**, 279 (1918).

³ Pinkus, *Ber.*, **31**, 31 (1898).

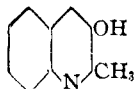
⁴ Nef, *Ann.*, **335**, 247 (1904).

⁵ Wohl, *Biochem. Z.*, **5**, 57 (1907).

⁶ Neuberg and Oertel, *ibid.*, **55**, 494 (1913). Neuberg and Rewald, *ibid.*, **71**, 144 (1915).

that so many different sugars, including pentoses, form acetol on distillation with sodium hydrogen carbonate. This has led us to the assumption that acetol must play a more important role in the intermediary metabolism of carbohydrates than is at present recognized.

Acetol, when heated with *o*-amino-benzaldehyde and a few cubic centimeters of potassium hydroxide solution, condenses to form 3-oxyquinaldine



which is readily recognized by its intense bluish fluorescence in sodium bicarbonate solution. This reaction is specific for acetol, in so far as is known. It is not given by pure methyl glyoxal or any of the following compounds which might be conceived by some rearrangement to give 3-oxyquinaldine with *o*-amino-benzaldehyde: aldol,⁷ acetone, acetaldehyde, aceto-acetic ester, crotonaldehyde,⁷ ethyl alcohol.

The following substances do not respond to this reaction under the conditions of this test, for they are not volatile from sodium bicarbonate solution: aceto-acetic acid, glycerol, β -hydroxybutyric acid, lactic acid.⁸ The property of yielding acetol with sodium bicarbonate seems to be quite general with most of the simpler carbohydrates and this reaction may be used as a method for detecting them in small quantities. We have obtained acetol by distillation of the following carbohydrates with sodium bicarbonate as shown by a positive test for 3-oxyquinaldine: arabinose, xylose,⁹ ribose,⁹ lyxose,⁹ glucose, fructose, mannose, galactose, glucosamine, lactose, sucrose, maltose, dextrin. On distillation in a like manner, starch and glycogen produced no acetol, nor could any be obtained from starch which had been oxidized for 48 hours with sodium pentacyano-aqua ferroate and oxygen and subsequent distillation.

The delicacy of this reaction has not been determined except for glucose, with which as small quantities as 5 mg. give a positive test. With the other carbohydrates, the test was carried out with 0.1 g. of the substance dissolved in 100 cc. of a 5% sodium hydrogen carbonate solution. We were unable to obtain a positive test for sucrose with less than 0.2 g., confirming the well-known fact that sucrose is much less readily broken up in alkaline solution than are the other disaccharides.

Experimental Part

A typical determination on a carbohydrate was carried out in the fol-

⁷ The aldol and crotonaldehyde were furnished to us by Mr. R. R. Read, of the Sheffield Laboratory of Organic Chemistry.

⁸ We have recently found that lactic acid and alanine when oxidized with ferrous oxide-peroxide yield pyruvic acid. This reaction will be studied further to determine whether it can be used for the quantitative determination of lactic acid.

⁹ Chemically pure ribose, xylose and lyxose were kindly furnished us by Dr. P. A. Levene of the Rockefeller Institute.

lowing way. To a solution of 0.1 g. of the sugar dissolved in 100 cc. of water was added 5 g. of solid sodium hydrogen carbonate, and this was distilled nearly to dryness. With the carbohydrates which are more readily attacked, the solution in the distilling flask very quickly turns brown and the distillate has a sweet odor. The residue in the flask after distillation has a strong typical caramel odor.

To the distillate were added 30 mg. of *o*-amino-benzaldehyde dissolved in a little alcohol, enough potassium hydroxide solution to give a distinctly alkaline reaction and a piece of porous plate to prevent bumping. This was evaporated over a free flame to about $\frac{1}{3}$ volume, cooled, and acidified with hydrochloric acid. Then solid sodium bicarbonate is added until the solution is alkaline to litmus. If one has a positive test, there is a strong bluish fluorescence,¹⁰ which can be seen in daylight but more readily in light of short wave length such as that yielded by the iron arc light. In order to confirm this test, it is necessary only to extract the 3-oxyquinaldine by shaking the solution several times with alcohol-free ether, dry the ether with a little sodium sulfate and then distil it. The white needle-like crystals of 3-oxyquinaldine which remain dissolve readily in a little alcohol and the fluorescence shows up strongly on the addition of water to the alcohol solution.

Summary

It has been found that all the simpler carbohydrates, including the pentoses, decompose on distillation with sodium hydrogen carbonate, yielding as one of their products the ketone alcohol, acetol, which is readily identified by a test described here. The identification of this alcohol under the conditions outlined, may serve as a qualitative method for the detection of the simpler carbohydrates.

The authors wish to express their appreciation to Professors Lafayette B. Mendel and Treat B. Johnson for their suggestions and criticisms during the progress of this work.

NEW HAVEN, CONNECTICUT

¹⁰ It is well known that anthranilic acid gives a blue fluorescence in aqueous solution. This acid cannot come into consideration in this test because we have never obtained a fluorescence in blank tests. We have proved that in case of a mixture of anthranilic acid and 3-oxyquinaldine, these substances can be separated by extraction with ether in NaHCO_3 solution. Only the 3-oxyquinaldine dissolves in the ether, while the amino acid remains in the water solution.