in long cream-colored needles from water containing a trace of sulfur dioxide. As already recorded by Gattermann, the product has no definite melting point.

In the acetylation, 2.3 g. of phoroglucin aldehyde in 100 cc. of dry ether containing 7.5 g. of anhydrous potassium carbonate and 15 cc. of acetic anhydride are worked up in the manner already described. The crude solid (3 g.) crystallizes from alcohol in thick colorless prisms which melt at 101°. The alcoholic solution gives no coloration with ferric chloride.

Anal. Subs., 5.100, 4.869 mg.: CO_2, 10.420, 9.930 mg.; H_2O, 1.98, 1.85 mg. Subs., 5.333, 5.930 mg.: 5.68, 6.37 cc. of *N*/100 NaOH. Calcd. for $C_{13}H_{12}O_7$: C, 55.71, H, 4.29; CH_3CO, 46.07. Found: C, 55.74, 55.64; H, 4.34, 4.25; CH_3CO, 45.82, 46.21.

The 2,4,6-triacetoxybenzylidenediacetate (I), obtained by the method of Pratt and Robinson, melted at 157°.

Anal. Subs., 4.979, 5.075 mg.: CO_2, 9.730, 9.930 mg.; H_2O, 2.06, 2.09 mg. Subs., 6.769, 6.181 mg.: 8.57, 7.81 cc. *N*/100 NaOH. Calcd. for $C_{13}H_{12}O_7$ (triacetylphloroglucin aldehyde): C, 55.71; H, 4.29; CH_3CO, 46.07. Calcd. for $C_{17}H_{18}O_{10}$ (2,4,6-triacetoxybenzylidenediacetate): C, 53.39; H, 4.71; CH_3CO, 56.27. Found: C, 53.30, 53.36; H, 4.63, 4.61; CH_3CO, 54.47, 54.36.

For the micro-acetyl estimations we are indebted to Dr. Arnulf Soltys and to Dr. M. Zacherl, which were carried out by them in Professor Pregl's laboratory, according to the Pregl–Soltys method.[14]

Summary

A general method for the acetylation of *o*-hydroxy-aldehydes is described. The acetylation is carried out with acetic anhydride and anhydrous potassium carbonate in the presence of ether, in which manner benzylidenediacetate formation is avoided.

BRISTOL, ENGLAND

[CONTRIBUTION FROM THE BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC., YONKERS, NEW YORK]

A METHOD FOR THE DETERMINATION OF PEROXIDASE ACTIVITY[1]

BY JOHN D. GUTHRIE

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A mixture of α-naphthol and *p*-phenylenediamine has frequently been used for both the qualitative and quantitative estimation of oxidase activity.[2] This depends on the oxidation of the reagent by atmospheric oxygen to form an indophenol, oxidase acting as the catalyst. In the estimation of peroxidase, hydrogen peroxide takes the place of oxygen in the reaction. The α-naphthol–*p*-phenylenediamine mixture has, however, usually been regarded as too sensitive for peroxidase, and other compounds

[14] Pregl, "Die Quantitative Organische Mikroanalyse," Berlin, Dritte Auflage, 1930, p. 216.

[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 13.

[2] H. M. Vernon, *J. Physiol.*, **42**, 402–432 (1911).

have been used for its estimation, especially pyrogallol.[3] It is true that the α-naphthol–*p*-phenylenediamine reagent is too sensitive for peroxidase when used near the neutral point, but in more acid solutions the reaction is sufficiently slow to be followed quantitatively. Furthermore, catalase is inactive in acid solutions and its interference is, therefore, avoided.

In a previous paper[4] the α-naphthol–*p*-phenylenediamine reagent was used in a study of peroxidase in potatoes that had been treated with chemicals that break the rest period. The method used in this work gave relative values, only determinations made at the same time being comparable. The procedure has now been improved so that determinations made at different times may be compared.

Preparation of Substrate.—Prepare a citrate buffer, P_H 4.5, by dissolving 21 g. of crystalline citric acid in 170 cc. of *N* sodium hydroxide and diluting to one liter. To 200 cc. of this add 200 cc. of water, 1 g. of *p*-phenylenediamine hydrochloride, and 20 cc. of 4% α-naphthol in 50% alcohol. Filter the solution.

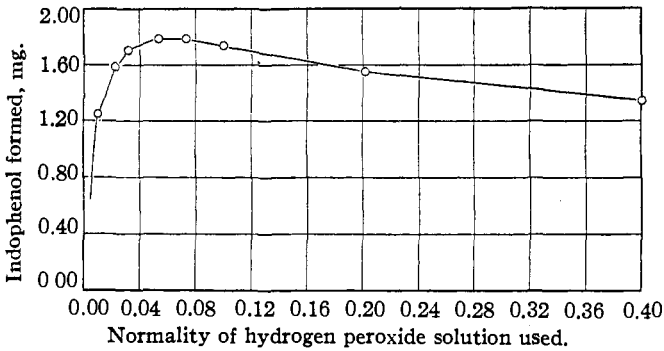

Fig. 1.—Showing the effect of using various hydrogen peroxide concentrations for the determination of peroxidase. Potato juice, 0.5 cc., was used in each determination.

The Method.—Place 25-cc. portions of the freshly prepared substrate in unlipped centrifuge tubes of about 80-cc. capacity. Place in a water-bath at 25° and allow to attain the temperature of the bath. Add 0.5 to 2.0 cc. of the juice or extract containing the enzyme, the amount depending on the peroxidase content. If the extract is very high in peroxidase, it will be necessary to make a known dilution before drawing the sample. Start the reaction by adding 5 cc. of *N*/20 hydrogen peroxide. When ten minutes have elapsed, stop the reaction by adding 5 cc. of 0.1% aqueous solution of potassium cyanide.

Remove from the bath and add 25 cc. of toluene. Stopper with a well fitting cork, shake vigorously and centrifuge. Pour off the clear layer of toluene, which now contains the indophenol, and compare it colorimetrically with the standard. It is usually desirable to run blank determinations on the reagents.

The Standard.—Prepare indophenol by adding 100 cc. of 2 *N* hydrogen peroxide and 2 cc. of 10% aqueous solution of $FeCl_3 \cdot 6H_2O$ to 800 cc. of the substrate. Allow to

[3] R. Willstätter and A. Stoll, *Ann.*, **416**, 21–64 (1918).

[4] F. E. Denny, L. P. Miller and J. D. Guthrie, *Am. J. Botany*, **17**, 483–509 (1930).

stand overnight. Filter off the precipitated indophenol and purify it by crystallization from a 1:1 mixture of absolute alcohol and toluene.

To make the standard, dissolve 50 mg. of this preparation in 50 cc. of a warm 1:1 mixture of absolute alcohol and toluene. Add toluene to make the total volume one liter. The standard keeps well.

A solution of iodine in chloroform, 2 g. per liter, is also a satisfactory standard. When set at 20 mm. in the colorimeter, it is equivalent to a solution of 75 mg. of the indophenol per liter.

Effect of Hydrogen Peroxide Concentration.—Since Willstätter and Stoll[3] have shown that high concentrations of hydrogen peroxide are harmful to peroxidase, determinations were made using hydrogen peroxide solutions of various normalities. These solutions were standardized with potassium permanganate. The results are shown in Fig. 1. The optimum amount of hydrogen peroxide to use is found to be 5 cc. of $N/20$ solution.

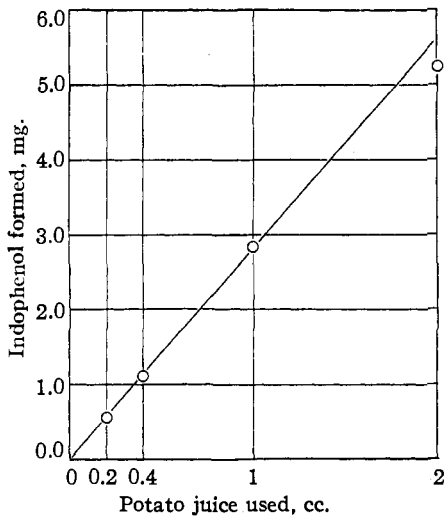

Fig. 2.—Showing the effect of various concentrations of peroxidase, obtained by the use of different volumes of potato juice.

Effect of Concentration of Enzyme.—The effect of using various amounts of potato juice is shown in Fig. 2. The enzyme activity is linear with the concentration of enzyme up to an activity of about 4.0. It is, therefore, recommended that extracts giving higher values be diluted before drawing the sample.

Accuracy of Method.—Potato juice was diluted one-quarter and determinations were made using 2 cc. of the diluted juice. The average indophenol value was 1.48 mg. with an average error of ± 0.03 mg.

Summary

A method for estimating peroxidase activity, based on the formation of indophenol from a mixture of *p*-phenylenediamine and α-naphthol in a citrate buffer of P_H 4.5, is described.

YONKERS, N. Y