pipetted into 5-ml. portions of the potassium ferricyanide-sodium carbonate reagent, and the amount of glucose was determined according to Hanes; a small correction for the enzyme and practically negligible corrections for the substrates were applied.

The data obtained are recorded in Tables III and IV and summarized in Table I. The methods for making the measurements in the methyl alcohol solutions were the same as those used for making the measurements in the aqueous solutions except that the substrate solution was made up by weighing out the sample of the glucoside in a 10-ml. volumetric flask, adding 5 ml. of methyl alcohol from a pipet, and then making to volume with the buffer solution. Two ml. of this solution was diluted with 1 ml. of enzyme for the hydrolysis.

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Summary

1. The relative ease of hydrolysis by the enzymes of almond emulsin, under the standard conditions of Helferich, has been determined for a number of alkyl β-D-glucosides belonging to the n-alkyl, cyclohexyl, and benzyl homologous series.

2. It is shown that for these series there is a pronounced tendency for the ease of hydrolysis to increase with the length of the aglucon chain.

3. For the n-alkyl β-D-glucosides, an increase in the chain length of the aglucon group beyond 7 or 8 carbons results in a decrease in the rate of hydrolysis. The possible implications of this decrease in the enzymic hydrolysis of polysaccharides and proteins are discussed.

4. The preparation and properties of n-amyl β-D-glucoside, n-heptyl β-D-glucoside, 2-cyclohexylethyl β-D-glucoside, and their tetraacetates are described for the first time. New measurements are reported for the rotations of the n-alkyl β-D-glucosides in water (from n-amyl to n-decyl) and for the tetraacetates in chloroform. The melting points of the same series have been remeasured, using a microscope equipped with a hot stage.

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The Action of Almond Emulsin on Populin and on Phenyl 2,4,6-Trimethyl-β-D-glucoside[1]

BY WILLIAM WARD PIGMAN AND NELSON K. RICHTMYER

Helferich and his co-workers[2] have demonstrated that the substitution of the hydroxyls of the pyranose ring of β-glucosides by methoxyl and other groups makes the substituted β-glucoside unhydrolyzable[3] by the β-glucosidase of almond emulsin. On the other hand, substitutions on the sixth carbon atom affect the enzymic hydrolysis of the glucosidic linkage in a measure dependent on the size of the group replacing the hydroxyl.[4]

In order to test these conclusions we decided to study the action of the β-glucosidase of sweet almond emulsin on two substituted glucosides which were available as a result of earlier investigations. One of these, populin, has been shown[5] to be o-hydroxymethylphenyl 6-benzoyl-β-D-glucoside (6-benzoylsalicin) and the other[6] to be phenyl 2,4,6-trimethyl-β-D-glucoside. It would be anticipated from the earlier work that the phenyl trimethyl-β-glucoside would not be hydrolyzed since the hydroxyls of the pyranose ring are substituted, while the populin, which is substituted by a benzoyl group only at the sixth position of salicin, might be hydrolyzed slowly.

The results, given in detail in the experimental part, agree with these predictions. Thus, in spite of the use of high enzyme concentrations, a hydrolysis time of seventeen days and a temperature of 37°, no hydrolysis of the phenyl trimethyl-

(1) Publication authorized by the Director of the National Bureau of Standards, and by the Surgeon General, U. S. Public Health Service.

(2) B. Helferich and O. Lang, Z. physiol. Chem., 216, 123 (1933); B. Helferich and S. Grünler, J. prakt. Chem., 148, 107 (1937).

(3) Previous to the work of Helferich, the term "unhydrolyzable" was used only in a qualitative sense. As used by the present writers, it refers to an enzyme efficiency of less than 10^{-6} (EE = $k/(g \times \log 2)$; k is the first-order reaction constant, $T = 30°$, substrate concn. = $0.052 M$, pH = 5.0, and g = grams of enzyme, of β-glucosidase value about 1.0, in 50 ml. of reaction mixture).

(4) B. Helferich, S. Grünler, and A. Gnüchtel, Z. physiol. Chem., 248, 85 (1937); W. W. Pigman, J. Research Natl. Bur. Standards, 26, 197 (1941).

(5) N. K. Richtmyer and E. H. Yeakel, THIS JOURNAL, 56, 2495 (1934).

(6) N. K. Richtmyer, ibid., 61, 1831 (1939).

β-D-glucoside could be observed (EE $< 10^{-5}$), while for the populin the small value of EE = 2 $\times$ 10^{-5} places this compound at the border of the unhydrolyzable class.

It is of interest to compare the rates of enzymic hydrolysis of the corresponding unsubstituted compounds. Thus the introduction of three methyl groups into the molecule of phenyl β-D-glucoside reduces the enzyme efficiency from 0.33[7] to less than 10^{-5}, while the benzoylation of salicin in the 6-position decreases the enzyme efficiency from a value of 1.7[7] or 1.5[8] for salicin to 2 $\times$ 10^{-5} for populin. The failure of populin to be hydrolyzed has been made the basis for the quantitative determination of salicin and populin in Salix.[9]

The results obtained for the phenyl trimethyl-β-D-glucoside, as well as those of Helferich, throw considerable doubt on the earlier claims[10] that methyl tetramethyl-β-D-glucoside is hydrolyzed by almond emulsin.

Experimental

The procedure used consisted in treating a solution of the substrate in Walpole's 0.2 M acetate buffer solution (pH, 5.0 at 18°) with a concentrated solution of sweet almond emulsin ("Rohferment" of Helferich,[11] of β-glucosidase value[12] 1.05) containing 0.1834 g. of enzyme in 10 ml. of solution. The solutions so prepared and blank solutions containing enzyme or substrate alone were kept at 37° for the desired time. The extent of hydrolysis was measured by titration with iodine in alkaline solution of the sugar and phenol produced. All the experiments were carried out in the presence of toluene. Additional details of the methods used are given in earlier publications.[13]

(7) B. Helferich, *Ergeb. Enzymforsch.*, **7**, 83 (1938).

(8) W. W. Pigman, *J. Research Natl. Bur. Standards*, **27**, 1 (1941).

(9) A. Kuhn and G. Schäfer, *Pharm. Ztg.*, **82**, 949 (1937).

(10) T. Purdie and J. C. Irvine, *J. Chem. Soc.*, **85**, 1049 (1904); J. C. Irvine and A. Cameron, *ibid.*, **87**, 900 (1905); *cf.* Kuhn and Schlubach, *Z. physiol. Chem.*, **143**, 154 (1925).

(11) B. Helferich, S. Winkler, R. Gootz, O. Peters, and E. Günther, *ibid.*, **208**, 91 (1932).

(12) R. Weidenhagen, *Z. Ver. deut. Zuckerind.*, **79**, Tech. Tl. 597 (1929).

(13) W. W. Pigman, *J. Research Natl. Bur. Standards*, **26**, 197 (1941); THIS JOURNAL, **62**, 1371 (1940).

Phenyl 2,4,6-Trimethyl-β-D-glucoside.—A solution made by adding 0.64 g. of substrate in 8 ml. of buffer solution to 4 ml. of enzyme solution was kept seventeen days at 37°. The solution then consumed 3.92 ml. of 0.1 N iodine solution. The enzyme alone consumed 3.85 ml. and the substrate alone 0.07 ml. The amount of iodine solution consumed by the products of hydrolysis was 0.00 ml.

Populin.—A suspension of 0.3047 g. of populin in 20 ml. of buffer solution was prepared and 10 ml. of the enzyme solution and 1 ml. of toluene were added.

A suspension kept for seven days at 37° consumed 12.57 ml. of 0.1 N iodine solution; the enzyme alone used 9.28 ml., and the corrected consumption was 3.29 ml.

A second suspension was kept at 37° for seventeen days. The enzyme and the substrate blanks, also kept for seventeen days, were mixed after this time and then kept at 37° for four hours. The reaction mixture consumed 15.02 ml. of 0.1 N iodine solution, while a mixed enzyme-substrate blank used 9.08 ml. The iodine consumption by the reaction products was then 5.94 ml. Although glucose, and presumably 6-benzoylglucose (which was not available), react quantitatively with iodine under these conditions, the salicyl alcohol (saligenin) does not. Therefore the extent of the hydrolysis was estimated by determining the amount of iodine consumed by a mixture of equal molar proportions of salicyl alcohol and glucose under the same conditions. A mixture of 0.0619 millimole each of salicyl alcohol and glucose required 5.94 ml., while 0.0337 millimole required 3.29 ml. For 100% hydrolysis, 0.781 millimole each of glucose and salicyl alcohol would be formed. At seventeen days (24,500 min.), the hydrolysis is 7.9%, $k = 1.5 \times 10^{-6}$, and EE = 1.6 $\times$ 10^{-5}. At seven days (10,000 min.), the hydrolysis is 4.3%, $k = 1.9 \times 10^{-6}$, and EE = 2.1 $\times$ 10^{-5}.

Summary

In confirmation of the earlier results of Helferich and co-workers for similar compounds, populin (6-benzoylsalicin) and phenyl 2,4,6-trimethyl β-D-glucoside were found to be unhydrolyzed by the enzymes of almond emulsin.