TABLE II

OSMOTIC PRESSURE OF ICSH SOLUTIONS AT 0°

pH	Protein concn./100 g. of buffer C, g.	Water pressure, p, cm.	C/P	Mol. wt.
4.60	0.990	5.55	0.178	41,000
4.58	1.090	6.35	0.172	39,800
4.60	0.925	6.10	0.168	39,000
			Average	40,000

It is of interest to note that the molecular weight of the hormone, as calculated from the analytical results previously[16] reported, is also approximately 40,000. It is assumed that the hormone consisted of two molecules of tryptophan and 10 molecules of tyrosine.

Comparison with Pig ICSH

Recently Shedlovsky, *et al.*,[2] isolated ICSH in chemically pure form from pig pituitary. The physical properties described by them differ considerably from those found for sheep ICSH. The pig ICSH is said to have a molecular weight of about 90,000 while that of sheep as described in this paper is 40,000. The isoelectric point of pig ICSH is stated to be 7.45 whereas that of sheep is 4.6.

The ICSH isolated from pig and from sheep pituitary glands also appear to differ considerably chemically. A pure pig ICSH which was kindly supplied by Dr. van Dyke was analyzed for carbohydrate and tryptophan content. It was found to contain approximately 2.0% carbohydrate and 3.8% tryptophan using the orcinol[17] and glyoxalic acid[18] methods, respectively. The sheep ICSH on the other hand contains 4.5% carbohydrate and 1.0% tryptophan. It is not unreasonable that hormones like other proteins isolated from different species may not be identical.

(16) Li, Simpson and Evans, *Science*, **92**, 355 (1940).

Summary

1. A modification of the method for isolation of sheep interstitial cell stimulating hormones (ICSH) is described.

2. The preparation behaves as a single substance in electrophoresis and in ultracentrifuge and solubility studies.

3. The isoelectric point of the sheep ICSH has been shown to be *p*H 4.6. The molecular weight has been determined to be 40,000, both from osmotic pressure studies and analytical data.

4. A comparison between sheep and pig ICSH has been made.

(17) Sørensen and Haugaard, *Biochem. Z.*, **260**, 247 (1933).

(18) Shaw and MacFarlane, *Canad. J. Research*, Sect. B., **16**, 351 (1938).

BERKELEY, CALIFORNIA RECEIVED SEPTEMBER 3, 1941

[CONTRIBUTION FROM THE NATIONAL BUREAU OF STANDARDS, U. S. DEPARTMENT OF COMMERCE, AND THE NATIONAL INSTITUTE OF HEALTH, U. S. PUBLIC HEALTH SERVICE]

The Influence of Structural Changes in the Aglucons on the Enzymic Hydrolysis of Alkyl β-D-Glucosides[1]

BY WILLIAM WARD PIGMAN AND NELSON K. RICHTMYER

A study of the effect of alterations in the sugar portions of glycosides has shown that "almond emulsin does not hydrolyze all glycosides but instead only the glycosides of naturally occurring sugars or of sugars which may be considered to be derived from these sugars by a simple substitution outside the pyranose ring."[2] This type of specificity may be termed "sugar specificity." Although slight structural or configurational changes in the sugar portion of hydrolyzable glycosides may inhibit completely the enzymic hydrolysis, considerable alterations may be made in the structures of the aglycons of hydrolyzable glycosides without preventing completely the enzymic hydrolysis. This type of specificity might be designated as "aglycon specificity." Quantitative studies of the "aglycon specificity" have been limited principally to the hydrolysis of β-glucosides by almond emulsin. Helferich and his co-workers,[3] in particular, have studied the effects of substitution in the aromatic nucleus of phenyl β-glucoside, while Veibel and his associates[4] have reported in considerable detail, although under somewhat different conditions, on the results of structural alterations in the aglucon groups of the alkyl β-glucosides. In the present investigation we have made a quantitative study of the action of the β-glucosidase of sweet almond emulsin on the *n*-alkyl β-D-glucosides[5] from the *n*-amyl to the *n*-nonyl glucoside, and on several

(1) Publication authorized by the Director of the National Bureau of Standards, and by the Surgeon General, U. S. Public Health Service (not copyrighted). Presented before the Division of Sugar Chemistry and Technology at the Atlantic City meeting of the American Chemical Society, September 8–12, 1941.

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

(3) B. Helferich, H. Scheiber, R. Streeck and F. Vorsatz, *Ann.*, **518**, 211 (1935); B. Helferich and C. P. Burt, *ibid.*, **520**, 156 (1935).

(4) S. Veibel and H. Lillelund, *Kgl. Danske Videnskab. Selskab, Math. fys. Medd.*, **17**, no. 6 (1940); *Z. physiol. Chem.*, **253**, 55 (1938).

(5) We are indebted to Dr. C. R. Noller of Stanford University for supplying us with samples of a number of the *n*-alkyl glucosides which have been used in this study.

members of the cyclohexyl and the benzyl homologous series of glucosides. The *n*-decyl and *n*-dodecyl glucosides, although available, were too insoluble in water to be measured under conditions comparable to those for the other glucosides.

TABLE I

SUMMARY OF ACTION OF ALMOND EMULSIN ON ALKYL β-D-GLUCOSIDES R-O-Gl

Aglucon Group (R)	Average EE	Relative EE
Methyl	0.036	1.00
n-Amyl	.47	13.1
n-Hexyl	.63	17.5
n-Heptyl	1.13	31.4
n-Octyl	1.05	29.2
n-Nonyl	0.72	20.0
Cyclohexyl	.39	10.8
Cyclohexylmethyl	.49	13.6
Cyclohexylethyl	.72	20.0
Phenyl	.33[a]	9.2
Benzyl	.48	13.3
Phenylethyl	.51	14.2
Phenylpropyl	.62	17.2

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

In Table I are summarized the results given in detail in the experimental part. In order to compare the ease of hydrolysis of the series of glucosides, a unit called the enzyme efficiency (EE) has been used. This unit,[6] a function of the velocity constant under strictly defined conditions, was introduced by Helferich and is called by him the "Wertigkeit."

In the *n*-alkyl series, the enzyme efficiency increases with the molecular weight of the aglucon and reaches a maximum for the heptyl and octyl glucosides, the nonyl glucoside exhibiting a definite decrease from the octyl glucoside.

The *n*-heptyl glucoside is hydrolyzed more than thirty times as fast as the methyl glucoside. The value 0.036 given for the enzyme efficiency of methyl β-glucoside is in good agreement with the value 0.034 reported previously by Helferich.[7] The ease of hydrolysis of the *n*-heptyl glucoside (EE, 1.13) compared with that of phenyl β-glucoside (EE, 0.33) contradicts the general impression[8] that the alkyl glucosides are hydrolyzed much more slowly than the aromatic glucosides. This is demonstrated also by the benzyl glucoside and its homologs. From phenyl to phenylpropyl β-glucoside, the enzyme efficiency increases steadily although any induced effect of the phenyl group decreases as the aromatic ring is removed from the glucosidic linkage. The enzyme efficiency thus increases as the aromaticity of the aglucon decreases. Additional information is furnished by the results obtained for the cyclohexyl β-glucoside and homologs. The members of this series are hydrolyzed slightly more readily than the corresponding members of the benzyl glucoside series. However, the most easily hydrolyzed glucosides known (*e. g.*, vanillin glucoside with EE = 137) are aromatic glucosides.

Figure 1 shows the enzyme efficiency plotted against the number of carbon atoms in the aglucon group, and includes the corresponding values for other β-glucosides as reported by Helferich[9] and by Veibel.[4] The data of Helferich are strictly comparable to the new data, but those of Veibel and Lillelund were determined under somewhat different conditions.[10]

The most striking features of Fig. 1 are: the maximum in the curve for the *n*-alkyl β-glucosides; the general tendency for the ease of hydrolysis to increase with increase in the chain length of the aglucon; and the exceptional behavior of the tertiary-alkyl β-glucosides. In addition, it may be observed that, of the compounds shown, the only series more readily hydrolyzable than the *n*-alkyl glucosides is that in which the aglucon is a glycol. The values for all of the compounds measured by Veibel and Lillelund are given except for the glucosides of the optically active butanols. These compounds are quite anomalous in their behavior and apparently require further investigation.

If the maximum illustrated in Fig. 1 for the *n*-alkyl β-glucosides is a general property of other homologous or structurally iterated series, it would seem to have important implications. As is well known, there are many enzymes which hydrolyze the same types of linkages but not the same compounds, *e. g.*, α-glucosidase and the amylases, the peptidases and the proteases. The

(6) EE = $k/(g \times \log 2)$, where k is the first-order reaction constant measured at 30°, for *p*H = 5.0 and for a glucoside concentration of 0.052 *M*; g = grams of enzyme (β-glucosidase value about 1.0) in 50 ml. of reaction mixture.

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

(8) E. F. Armstrong and K. F. Armstrong, "The Carbohydrates," Longmans, Green and Co., New York, N. Y., 1934, p. 215.

(9) B. Helferich and R. Hiltmann, *Ann.*, **531**, 160 (1937); B. Helferich and W. Göller, *Z. physiol. Chem.*, **247**, 220 (1937).

(10) To obtain comparable data, the values reported by Veibel and Lillelund have been interpolated graphically at a concentration of 0.052 *M*. The probable assumption was then made that for the other glucosides the same ratio exists as that between the interpolated value for the *n*-butyl β-glucoside and the EE measured by Helferich and Göller for the same compound. In the case of several glucosides the necessary data were not available (notably the amyl glucoside) and the comparison was made using the values of Veibel and Lillelund for 0.04 *M* substrate concentrations.

differences in the actions of these enzymes may consist in a different size or structure for the aglycon group at the optimal region for hydrolysis (or synthesis). Thus cellulose would lie far to the right on the above figure, and presumably would be hydrolyzed only with extreme slowness by β-glucosidase. Correspondingly, the simple β-glucosides would fall far to the left on a curve representing the cellopolyose series. The difference between enzymes such as trypsin and pepsin might be explained as a difference in the position of the optimal region for hydrolysis. Similarly, the Weidenhagen classification of enzymes may represent groups of enzymes hydrolyzing the same types of linkages (*e. g.*, a group of β-glucosidases) but requiring different optimal structures for the aglycon. Perhaps the checking of enzymic syntheses at certain degrees of polymerization and the partial rather than the complete hydrolysis of polysaccharides and proteins by enzymes may be due, at least in part, to the same cause. Unfortunately, the experimental verification for other homologous series is complicated by the diminishing solubility which often accompanies the increasing size of the aglycon.

The existence of a maximum in the enzyme efficiencies of the *n*-alkyl glucosides seems certain, although the exact position is uncertain, since the difference between the EE values for the *n*-heptyl and *n*-octyl glucosides is close to the experimental error. The results given for the *n*-hexyl, *n*-octyl, and *n*-nonyl glucosides represent the averages of two sets of measurements carried out at quite different times on two independently prepared sets of compounds.

In order to obtain some information on the cause of the maximum, experiments were made to investigate the possibility that this effect might be due to the separation of a second phase which appears during the hydrolysis of the *n*-octyl and *n*-nonyl glucosides. This second phase consists of the alcohol liberated during the hydrolysis. Although this explanation seems improbable, since the amount of the second phase formed is small and since good first-order reaction constants are obtained even when a second phase is present, an attempt was made to carry out the reaction in a single phase by using a solution containing 33% of methyl alcohol by volume. As shown in detail in Table IV, in this solvent the reaction proceeds less than one-tenth as rapidly as in aqueous solution, and in contrast to the good constants obtained for the reactions in aqueous solutions, the constants for the reactions in the solutions containing methyl alcohol exhibit a rapid decrease as the hydrolysis proceeds. This solvent also was found not to dissolve the alcohols sufficiently well to prevent a second phase from separating.

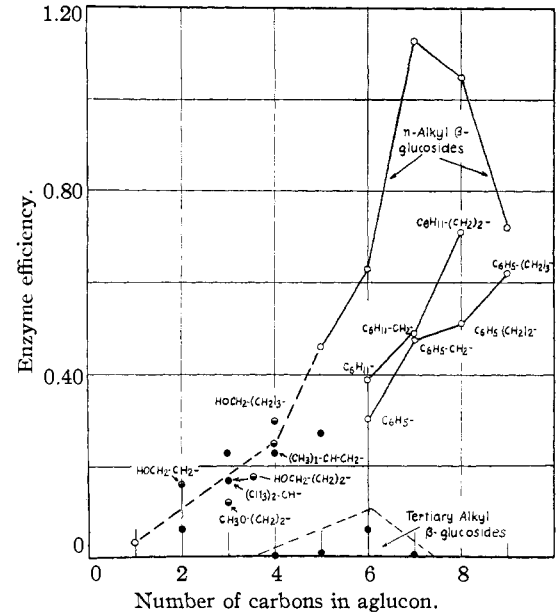

Fig. 1.—Comparison of EE with the number of carbons in the aglucon of the β-glucoside. Open circles represent new data reported in this publication. Filled circles indicate data of Veibel and Lillelund, and half-filled circles are data of Helferich and co-workers. The circles for which formulas are not given belong to the *n*-alkyl series.

Although the enzyme efficiencies are usually calculated from first-order reaction constants obtained in the interval between 20 and 40% hydrolysis, several experiments were carried out over longer intervals to investigate the applicability of the first-order equation. As shown in Table III, both the cyclohexyl and the nonyl β-glucosides gave fairly good values for the reaction constant during the entire course of the reaction.

Two experiments were carried out to investigate the decrease of the reaction constant in the aqueous methyl alcohol. As shown in Table IV, neither a denaturation of the enzyme by the methyl alcohol nor an inhibition by the alcohol formed during the hydrolysis is sufficient to explain the decrease in the reaction constants.

A mechanism has been suggested previously for the hydrolysis of glycosides by the glycosidases.[11] According to this mechanism there are two areas of attachment between the enzyme and glycoside

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

involved in the formation of the intermediate adsorption complex. The aglycon group is attached to one area on the enzyme and the sugar radical to the other area. It would be expected that increasing the number of carbons in the aglycon would increase the number of adsorption bonds between the aglycon group and the enzyme and thus facilitate the hydrolysis. According to this view, however, the rate would not increase indefinitely, but at some point the progressively decreasing dissociation from the enzyme of the products of hydrolysis would tend to inhibit the reaction. In the *n*-alkyl glucoside series the maximum might be explained as the region in which the benefits of good adsorption of the aglycon alkyl group are balanced by the disadvantages of reduced dissociation from the enzyme of the products of hydrolysis. The slow hydrolysis of the tertiary-alkyl β-glucosides would seem to be due to the lack of a hydrogen on the aglycon carbon forming the glucosidic linkage. As this carbon would not be adsorbed on the enzyme, the activation energy would be distributed over several bonds. These explanations will be developed in more detail in later publications.

Experimental

The action of emulsin has been studied with twelve β-glucosides. Of these, the *n*-amyl glucoside was listed once in a table by Veibel and Lillelund,[12] but neither the glucoside nor its acetate was described. The *n*-hexyl, octyl, nonyl, and decyl glucosides and their acetates have been described by Noller and Rockwell.[13] The *n*-hexyl β-glucoside had been synthesized previously by Vintilescu, Ionescu and Solomon,[14] and its acetate was prepared first by Pacsu.[15] Inasmuch as the rotations reported by Noller and Rockwell were determined in methyl alcohol solutions, we have measured them in the preferred solvents, water and chloroform, for the glucosides and their acetates, respectively. The data for these glucosides, including the new amyl, heptyl, and cyclohexylethyl derivatives, are recorded in Table II. The other glucosides used were samples of those described previously.[16]

The *alkyl tetraacetylglucosides* were prepared, in general, by shaking overnight a mixture of 0.1 mole of tetraacetyl glucosyl bromide,[17] 0.5 mole of the appropriate alcohol, 0.2 mole of silver oxide, 0.5 mole of "Drierite," and 250 ml. of dry benzene. The solution was filtered through a layer of "Filter-cel," freed from benzene and excess alcohol by distillation with steam *in vacuo*, and the residue crystallized to constant rotation from aqueous methyl or ethyl alcohol. The yields, in general, were 60 to 75% of the theoretical. The crystalline appearance varied from the large prisms of the amyl compound to the thin, waxy plates of the decyl compound; the hexyl, heptyl, octyl, nonyl and cyclohexylethyl glucoside acetates were prismatic needles, tending to become finer needles, in that order.

TABLE II

PROPERTIES OF THE ALKYL β-GLUCOSIDES AND THEIR TETRAACETATES

	Glucoside M. p., °C.[a]	Glucoside $[\alpha]^{20}D$ in H_2O[b]	Acetate M. p., °C.[a]	Acetate $[\alpha]^{20}D$ in $CHCl_3$[c]
n-Amyl[d]	91.5–93	−36.3	45.2–45.5	−22.1
n-Hexyl	90–92	−34.5	50.5–51.5	−20.2
n-Heptyl[e]	74–77[f]	−34.2	66.0–68.5	−20.5
n-Octyl	62–65[f]	−34.0	62.8–63.0	−20.5
n-Nonyl	67.5–70[f]	−34.4	40.7–41.5	−19.5
n-Decyl	73–74[f]	−28.3[g]	49.5–51.0	−19.5
2-Cyclohexylethyl[h]	99–101	−32.3	75.0–75.5	−21.1

[a] Measured by the use of a microscope with hot stage. [b] Concentration 4–5%. [c] Concentration 4%. [d] *Anal.* Glucoside, calcd. for $C_{11}H_{22}O_6$: C, 52.78; H, 8.86. Found: C, 52.5; H, 8.6. Acetate, calcd. for $C_{19}H_{30}O_{10}$: C, 54.54; H, 7.23. Found: C, 54.7; H, 7.1. [e] *Anal.* Glucoside, calcd. for $C_{18}H_{26}O_6$: C, 56.09; H, 9.42. Found: C, 55.9; H, 9.2. Acetate, calcd. for $C_{21}H_{34}O_{10}$: C, 56.49; H, 7.68. Found: C, 56.6; H, 7.6. [f] Forms an anisotropic liquid at the melting point given. [g] Rotation in methyl alcohol. [h] The acetate was prepared by Miss Marion H. Armbruster at Bryn Mawr College in 1931. *Anal.* Glucoside, calcd. for $C_{14}H_{26}O_6$: C, 57.91; H, 9.03. Found: C, 57.7; H, 9.1. Acetate, calcd. for $C_{22}H_{34}O_{10}$: C, 57.63; H, 7.47. Found: C, 57.6; H, 7.6.

The *alkyl glucosides* in Table II were prepared by catalytic deacetylation of the corresponding acetate with barium methylate in boiling methyl alcohol, and crystallized from acetone solution, often by the addition of petroleum ether. The glucosides were then recrystallized several times from acetone, which seems a better solvent than the ethyl acetate used by previous workers. For the octyl glucoside, particularly, the temperature at which the crystallization takes place is important and should be 20° or less. The octyl and nonyl glucosides are hygroscopic under humid conditions.

The melting points of the glucosides recorded in Table II were measured by the use of a microscope equipped with a hot stage.[18] Preliminary measurements with the ordinary apparatus, using a capillary tube, had given unsatisfactory results and it should be noted that Noller and Rockwell had reported melting point ranges of about 55° for several of these compounds. Their explanation, that this is due to the formation of liquid crystals, seems to be correct. When the crystals are observed through a microscope, it is seen that they melt rather sharply but tend to keep their original outline and the liquid shows double refraction of polarized light. Since the crystals melt at rather a low temperature, it would seem that the phenomenon might be explained on the basis of a high viscosity and a low surface tension of the melt.

(12) S. Veibel and H. Lillelund, *Z. physiol. Chem.*, **253**, 62 (1938).

(13) C. R. Noller and W. C. Rockwell, THIS JOURNAL, **60**, 2076 (1938).

(14) I. Vintilescu, C. N. Ionescu and M. Solomon, *Bul. soc. chim. România*, **17**, 279 (1935).

(15) E. Pacsu, THIS JOURNAL, **52**, 2563 (1930).

(16) N. K. Richtmyer, *ibid.*, **56**, 1633 (1934).

(17) Preliminary attempts to prepare tetraacetyl *n*-hexyl glucoside from tetraacetyl glucosyl chloride were unsuccessful.

(18) We wish to thank Dr. William Stanton of the University of Maryland for his help and advice in making these measurements, and to thank Dr. N. L. Drake for making the apparatus available.

Enzyme Solution.—A 1-g. portion of sweet-almond emulsin (a sample of "Rohferment" furnished through the courtesy of Prof. B. Helferich[19]) was rubbed in a mortar with 100 ml. of water, the solution was chilled in the refrigerator for one hour, centrifuged, and the clear supernatant liquid was filtered. The residue from 10 ml. of solution weighed 0.0722 g. in one set of experiments and 0.0739 g. in a second set. The β-glucosidase value[20] was 1.05. For convenience in measuring the extent of hydrolysis of the glucosides described in this paper, 10-ml. portions of the above emulsin solution were diluted to 50 ml. with water, and further mention of "the enzyme solution" refers to this diluted, approximately 0.15%, solution of "Rohferment." The enzyme concentration in the reaction mixture was about 0.5 mg. per ml.

TABLE III

ENZYMIC HYDROLYSIS OF β-D-GLUCOSIDES[a]

T, 30° C.; *p*H, 5.0; substrate concn., 0.052 *M*

Time, min.	% Hydrolysis		$k^b \times 10^3$	EE[c]
	n-Hexyl β-D-glucoside			
25.5	22.0		4.23	
32	26.5		4.20	
37	30.7		4.31	
41.7	34.9		4.47	
∞	100	Av.	4.30[d]	0.59
	Inhibition of the *n*-hexyl glucoside by sorbitol[e]			
40	25.7		3.22	
121	61.4		3.42	
	n-Nonyl β-D-glucoside			
10.5	9.8		(4.27)	
20	18.9		4.55	
30	28.0		4.77	
45	39.3		4.82	
60	48.4		4.79	
90	64.2		4.96	
120	75.4		5.08	
180	86.1		4.76	
304.5	96.8		(4.91)	
∞	100	Av.	4.82[d]	0.66
	Cyclohexyl β-D-glucoside			
20	11.0		(2.53)	
40	21.6		2.64	
60.5	30.7		2.63	
85	41.2		2.71	
129	56.1		2.77	
181	67.2		2.67	
250	79.7		2.77	
300	84.0		2.65	
360	90.2		(2.80)	
∞	98	Av.	2.69[d]	.37

[a] The experimental data have been omitted except for a few representative examples. In most cases at least two sets of experiments were carried out at quite different times. The averages given in Table I and in Fig. 1 have been derived from data additional to those given in this table. [b] Calculated using minutes and common logarithms. [c] EE = enzyme efficiency = $k/(g \times \log 2)$; g = grams of enzyme in 50 ml. of reaction mixture. [d] g = 0.0241 g. of enzyme in 50 ml. of reaction mixture. [e] A solution was prepared by making up 0.0528 g. of *n*-hexyl glucoside and 0.0775 g. of sorbitol to a volume of 2.56 ml. To 2 ml. of this solution was added 1 ml. of enzyme solution.[d]

TABLE IV

ENZYMIC HYDROLYSIS IN AQUEOUS METHYL ALCOHOL (33% BY VOLUME)[a]

T, 30° C.; 0.0246 g. of enzyme in 50 ml. of reaction mixture; substrate concn., 0.052 *M*.

Time, min.	% Hydrolysis	$k^b \times 10^4$
	n-Heptyl β-D-glucoside	
89	13.0	6.80
120	16.2	6.40
204	18.9	4.46
296	20.7	3.40
	Action of methyl alcohol on the enzyme[c]	
88.9	12.8	6.69
204.4	18.9	4.58
297.4	20.8	3.40
	Inhibition by *n*-heptyl alcohol[d]	
120	14.6	5.71
200	19.2	4.63
300	20.9	3.39

[a] To a weighed amount of the substrate in a 10-ml. flask was added 5 ml. of methyl alcohol. The solution was made to volume with 0.2 *M* acetate buffer (*p*H, 5.0). To 2 ml. of this solution was added 1 ml. of the enzyme solution. [b] Calculated using minutes and common logarithms. [c] The enzyme, in 33% methyl alcohol solution, was kept for 18 hours at 30° before the addition of the solid *n*-heptyl glucoside. [d] *n*-Heptyl alcohol, in quantity equivalent to that produced by 100% hydrolysis, was added to the original heptyl glucoside solution (see footnote *a*) before the enzyme was added.

Substrate Solutions.—We have adhered to the "standard concentration" adopted by Helferich and co-workers for hydrolysis measurements, namely, that the amount of glucoside be molecularly equivalent to 40 mg. of anhydrous phenyl glucoside in 2 ml. of approximately 0.2 *N* acetate buffer solution (*p*H, 5.0 at 18°). The concentration of the reaction mixture obtained by adding 1 ml. of the enzyme solution thus is 0.052 molal.

Hydrolysis Measurements.—The extent of hydrolysis of the β-glucosides by emulsin was measured conveniently by determining the amount of glucose present at any given time with the aid of Hanes' modification[21] of the Hagedorn–Jensen micro-method[22] for reducing sugars. Substrate and enzyme were brought to temperature in a bath at 30.0 ± 0.1°. At zero time the desired amount of enzyme solution was added to twice its volume of substrate solution in a glass-stoppered test-tube. After measured intervals, 1-ml. (or smaller) portions of the reaction mixture were

(19) B. Helferich, S. Winkler, R. Gootz, O. Peters and E. Günther, *Z. physiol. Chem.*, **208**, 91 (1932).

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

(21) C. S. Hanes, *Biochem. J.*, **23**, 99 (1929).

(22) H. C. Hagedorn and B. N. Jensen, *Biochem. Z.*, **135**, 46 (1923).

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.

One of the authors (N. K. R.) desires to thank the Chemical Foundation of New York for a Research Associateship. The authors also express their indebtedness to Mr. Kenneth Fleischer and Dr. Arthur T. Ness for carrying out the microanalyses.

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.

WASHINGTON, D. C.
BETHESDA, MD. RECEIVED OCTOBER 7, 1941

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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^{-5} (EE = $k/(g \times \log 2)$; k is the first-order reaction constant, T = 30°, substrate concn. = 0.052 *M*, *p*H = 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).