Det Kgl. Danske Videnskabernes Selskab.
Mathematisk-fysiske Meddelelser. **XVII**, 6.

ENZYMIC HYDROLYSIS OF GLUCOSIDES
III. HYDROLYSIS OF THE 5 BUTYL-β-d-GLUCOSIDES

BY

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1940

Printed in Denmark.
Bianco Lunos Bogtrykkeri A/S.

INTRODUCTION

In continuation of previous work we have examined the enzymic hydrolysis of all 5 butyl-β-d-glucosides. Already in our paper from 1937 (1) we pointed out that it seems natural to us to refer the velocity of hydrolysis not to the amount of emulsin present in the solution, but to the amount of emulsin actually connected with the substrate. It will not, probably, be possible to give the final proof of the correctness of this aspect till the molecular weight of the catalyst β-glucosidase is known, so that its molar concentration may be taken into consideration, thus allowing a calculation of the molar concentration of the assumed complex substrate-enzyme and consequently a determination of the real monomolecular constants of velocity for the different glucosides. As it must be assumed, however, that the effect of this calculation will be the same for all the glucosides, so that all constants, referred to the amount of β-glucosidase connected with the substrate, are to be multiplied by the same factor M, M being the molecular weight of β-glucosidase, it seems likely that the relative values will not be changed, so that the constants determined in this series of papers will preserve their relative values, even if the molecular weight of the enzyme is introduced in the calculation of the constants.

We have shown that it is possible to take into consideration the inhibiting effect of the products of hydrolysis, at

all events as far as the inhibition in question is a competitive one; and the calculations carried out for the glucosides examined have proved that the inhibition caused by glucose and the alcohols in question is to a very great extent competitive. From the calculation of k_3, the monomolecular velocity-constant of hydrolysis of the enzyme-substrate-complex, the inhibition having been taken into consideration, Veibel and Eriksen (1) derived the expression

$$[1] \qquad k_3 = k_{obs} \cdot c/X \cdot e\,(\text{sal. f.}),$$

where k_{obs} is the directly observed velocity constant at the glucoside-concentration c and X is the fraction of the enzyme-preparation actually bound to the substrate. X may be calculated by means of the expression

$$[2] \qquad X = a \cdot \frac{(1-b)\,(1-c)}{1-ab-ac-bc+2\,abc},$$

a, *b* and *c* being the fractions of emulsin bound to glucoside, glucose, and alcohol respectively, if these substances alone were present in the solution. Within the limits of the experiment the expression [1] proved to be correct. Later, Veibel and Lillelund (2) have shown that the calculation of X may be simplified, as the expression [2] can be transcribed to

$$[3] \qquad X = \frac{(c-x)}{K_m + (K_m/K_{m_1} + K_m/K_{m_2})\,x + (c-x)},$$

where K_m, K_{m_1} and K_{m_2} are the dissociation constants of the compounds enzyme-glucoside, enzyme-glucose and enzyme-alcohol respectively.

A further simplification of the calculation of k_3 is possible.

VEIBEL and ERIKSEN (1) have shown that if the inhibiting effect of the products of hydrolysis is negligible, k_3 may be calculated from the expression

$$[4] \qquad k_3 = k'_{obs}\,(K_m + c)/e\,(\text{sal. f.}),$$

whereas by introducing the expression [3] for X in [1] we get

$$[5] \quad k_3 = k_{obs}\,(K_m + c + (K_m/K_{m_1} + K_m/K_{m_2} - 1)\,x)/e\,(\text{sal. f.}),$$

which is of the same form as [4], but in [5] the inhibiting action of the products of hydrolysis has been taken into consideration.

This expression is, in our opinion, more convenient than the expression

$$k_3 = (k'_{obs}\,(K_m + (K_m/K_{m_1} + K_m/K_{m_2})\,c)/e\,(\text{sal. f.}) - (K_m/K_{m_1} + K_m/K_{m_2} - 1)\,x/t)/e\,(\text{sal. f.})$$

commonly used for the calculation of k_3 in cases of inhibition caused by the products of hydrolysis. (For the signification of k'_{obs}, see p. 7).

If an inhibiting substance with the affinity-constant (the dissociation constant of the compound enzyme-substrate) K_{m_h} is present in the concentration h, its inhibition may be taken into account by adding the number $h \cdot K_m/K_{m_h}$ to the factor in paranthesis, and we get

$$[6] \quad k_3 = \frac{k_{obs}\,(K_m + c + h \cdot K_m/K_{m_h} + (K_m/K_{m_1} + K_m/K_{m_2} - 1)\,x)}{e\,(\text{sal. f.})}.$$

The complete description of the hydrolysis of a given enzyme may, therefore, be obtained if the constants K_m,

K_{m_1} and K_{m_2} are known. In the expressions [3]—[6] c is the initial concentration of glucoside and x is the mean concentration of the products of hydrolysis at the two points between which k_{obs} is calculated. This calculation is most advantageously carried out from point to point.

The constants are all of them made comparable by division by e (sal. f.), e being the amount of emulsin (in grams) present in 50 ml of the reaction mixture and sal. f. the enzymic force of the enzyme preparation used. We usually express the enzymic force as sal. f., as proposed by Josephson (3), but by means of the following system of equations

$$\beta\text{-Glucosidasewert} = 1/\text{Zeitwert} = \text{sal. f.}/\log 2 =$$
$$= k_{obs\,50\%}/e \cdot \log 2$$

other units than sal. f. may be introduced for the indication of the enzymic force.

The four units mentioned above are all by definition connected with the examination of the hydrolysis of salicin-solutions of the molar concentration 0.139. A more independent determination of the enzymic power has been proposed by Veibel and Lillelund (2), who by "salicin-value" denote the value $10^2 \cdot k_3$ for salicin, 1 g of combined emulsin being present in 50 ml solution, which can be determined by means of any salicin-concentration or by means of solutions of other glucosides, provided that proportionality factors, different for each glucoside, have been determined.

For the determination of K_m we have in our first papers (1) used a graphical method indicated by Lineweaver and Burk (4), making use of the initial velocity of hydrolysis

for solutions of different substrate concentration. VEIBEL (5) has shown that the velocity constants may be used instead of the initial velocities, and we now proceed to make use of the expression [4] in the form

$$[7] \qquad 1/k'_{obs} \cdot \text{const.} = K_m + c,$$

k'_{obs} being the velocity constant calculated not between two neighbouring points but from the time 0 to the time t. This expression is sufficiently correct, when only experiments the degree of hydrolysis of which is below some 25 % are used for the calculation of k'_{obs}. The inhibition caused by the products of hydrolysis is not then generally so great as to seriously compromise the constants.

In the calculation of K_{m_1} and K_{m_2} as well it is, as shown by VEIBEL (5), possible to use the velocity constants instead of the initial velocities. The affinity constant K_{m_h} of an inhibiting substance may be calculated from the expression

$$[8] \qquad K_{m_h} = \frac{K_m \cdot h}{(K_m + c)\,(k/k_h - 1)},$$

where K_m is the affinity constant of the glucoside, c its molar concentration, h the molar concentration of the inhibiting substance and k and k_h the directly observed velocity constants of the not inhibited and the inhibited system.

Experimental Part.

Substrates. The 5 substrates examined in this paper were: n-butyl-β-d-glucoside, iso-butyl-β-d-glucoside, l-methyl-ethyl-carbinol-β-d-glucoside, d-methyl-ethyl-carbinol-β-d-glucoside and trimethyl-carbinol-β-d-glucoside. The pre-

paration and the properties of these glucosides have been described by Veibel and Lillelund (6), and we may therefore confine ourselves to giving the values of the physical constants.

	n-Butyl-carbinol-glucoside	iso-Butyl-carbinol-glucoside	l-Methyl-ethyl-carbinol-glucoside	d-Methyl-ethyl-carbinol-glucoside	Trimethyl-carbinol-glucoside
M. P.	68—69°	112—113°	75—76°	116—117°	164—166°
$[\alpha]_D^{20}$ Water ...	—36.9°	—39.9°	—44.5°	—32.1°	—19.0°
$[\alpha]_D^{20}$ K_2CO_3 ...	—38.1°	—41.6°	—46.4°	—33.3°	—20.3°

The values $[\alpha]_D^{20}$ K_2CO_3 mean the values of $[\alpha]_D^{20}$ at p_h 10.5—10.6, the concentration of hydrogen ion of the samples, when the enzymic action has been stopped by the addition of samples of 5 ml reaction-mixture of p_H 4.4 to 1 ml of a 20 % potassium carbonate solution (Veibel and Eriksen, (7)). On the average, the specific rotation of glucose and of glucosides is moved 3—4 % to the left when determined at p_H 10.5—10.6 instead of by neutral reaction, and the figures above show this to be correct for the glucoside of the tertiary alcohol examined, the rotation of which is moved 6—7 %, as well as for those of primary or secondary alcohols examined before.

Technique. The technique employed is the same as that used in the previous papers. Standard substrate concentration is 0.0400 m, standard p_H 4.40, obtained by means of an acetate buffer with a total concentration of acetate ion in the reaction-mixture 0.030 m. Samples of 5 ml are withdrawn at the times required, added to 1 ml of a 20 % K_2CO_3-solution and kept at least 3 hours before the determination of the rotation. All values given in the tables below are corrected for the alterations of rotation taking

place in glucose-solutions when they are kept at p_h 10.5—10.6 (7).

The constants, except those used in the calculation of K_m, are calculated from point to point. All constants are calculated with the minute as unit of time and with logarithms to base 10. The molar concentration of glucoside and of the products of hydrolysis are calculated for each sample, thus permitting the calculation of k_3 by means of equation [5], when the K_m-values have been determined. The difference between two readings may in some instances drop to 0.035—0.040°, and the experimental error on the values of k_3 is therefore in such cases considerable, but the consecutive values are dependent on each other in such a way that too small a value of one constant causes either the foregoing or the following constant to be found too great, and the average value will therefore be approximately correct.

e is determined in each experiment by evaporation of the same amount of the emulsin-solution as that used in the experiment and drying the residue to constant weight at 105°. Sal. f. has for the emulsin-preparations used in the experiments mentioned here been determined by means of a 0.139 m salicin-solution, as indicated by WEIDENHAGEN (8).

The K_m-value for a glucoside is calculated from the k'_{obs}-values at 6 different glucoside-concentrations, chosen so as to make the variation in the amount of emulsin bound to the substrate as great as possible. The reaction is followed polarimetrically, and as the accuracy of the readings does not surpass 0.005°, the concentrations which it is possible to make use of have a lower limit at about 0.01 molar solution, the total change of rotation for a 0.01 m solution

being approximately 0.30° and the degree of hydrolysis in these experiments being limited to 25% or, for the most diluted solutions, to 50%. As 4 readings are usually taken in each experiment, a total variation of 0.15° means that the difference between two readings is only 0.035—0.040°, and the experimental error in the values of k'_{obs} may be considerable for 0.01 m glucoside-solutions, but already at 0.02 m solutions the experimental error due to the inexactness of the readings should not surpass some few per cent.

6 corresponding values of k'_{obs} and c, introduced in equation [7], determine a straight line, the intercept of which on the abscissa-axis is $-K_m$. It is seen from the curves, e. g. for n-butyl-glucoside (p. 12) that glucoside concentrations higher than some 0.10 m are to be avoided, the k'_{obs}-values at higher concentrations being somewhat greater than calculated from equation [7].

K_{m_1}, the affinity constant for glucose, has in previous examinations been found to be 0.18, this value agreeing with values found by other investigators, e. g. JOSEPHSON (3). Here, we have determined K_{m_1} in the case of n-butyl- and of iso-butyl-glucoside and have found values which within the limits of the experiment are identical herewith. We have not hesitated, therefore, to use this value also in cases where the direct determination of K_{m_1} has not been possible.

In the two cases where the determination has been carried out, toluene has, as usual, been added to the solutions in order to prevent the growth of microorganisms, and it has therefore been possible to determine the toluene-effect (VEIBEL (9)) for butyl- and iso-butyl-glucoside.

K_{m_2}, the affinity-constant of the alcohol, has been determined in the usual way (the same way which was used

in the case of K_{m_1}), by comparing the values of k_{obs} of a glucoside-solution at standard conditions and of solutions which besides glucoside contain the alcohol in question in the concentrations 0.01, 0.02, 0.04, 0.08 or 0.12 m. Equation [8] then allows the determination of K_{m_2}, and, in our opinion, the mean value of the 5 determinations is rather accurate.

In the cases of l- and d-methyl-ethyl-carbinol it has been necessary to proceed in a somewhat different manner, and the determination, which will be described later, is not so reliable as in the other instances.

1. n-Butyl-β-d-glucoside.

Determination of K_m.

Table I.

a. 30°. e = 0.0920.

sal. f. = 0.078.

c	$10^4 \cdot k'_{obs}$	e (sal. f.)/k'_{obs}
0.0100	60.57	1.18
0.0200	33.22	2.16
0.0400	19.56	3.67
0.0800	12.52	5.73
0.1200	9.41	7.63
0.1600	7.87	9.12

Table II.

b. 20°. e = 0.1014.

sal. f. = 0.078.

c	$10^4 \cdot k'_{obs}$	e (sal. f.)/k'_{obs}
0.0200	17.75	4.46
0.0400	12.44	6.36
0.0800	8.14	9.72
0.1600	5.48	14.43
0.2400	4.41	17.93
0.3200	3.45	22.93

In fig. 1 are plotted not only the values indicated in the experiment described above but also values from another experiment in which the glucoside-concentration varied between 0.0200 m and 0.3200 m (30°). It is seen from the figure that at low glucoside concentrations the values of e (sal. f.)/k'_{obs} agree well with the straight line determined in the first experiment, whereas at higher concentrations

the k'_{obs}-values exceed the values demanded by the straight line.

The value of K_m, both at 30° and at 20°, is 0.031.

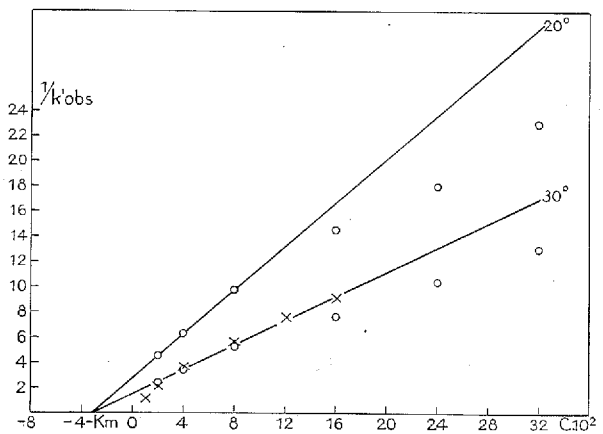

Fig. 1.

c. Determination of K_{m_1}, the affinity constant of glucose.

Table III.

30°. Glucoside 0.0400 m. $K_m = 0.031$. e = 0.0763. sal. f. = 0.078. 1 ml toluene to 50 ml solution.

$c_{glucose}$	$10^4 \cdot k_{obs}$	k/k_h	$K_{m_1} = \frac{K_m \cdot c_{glucose}}{(K_m + c)(k/k_h - 1)}$
0.00 m	21.7	—	—
0.01 m	20.8	1.040	0.109
0.02 m	20.2	1.071	0.123
0.04 m	20.1	1.079	0.221
0.08 m	19.6	1.157	0.223
0.12 m	19.1	1.198	0.265
			average... 0.19

d. Determination of K_{m_2}, the affinity constant of n-butyl-alcohol.

Table IV.

α. 30°. Glucoside 0.0400 m. $K_m = 0.031$. $e = 0.0763$. sal. f. = 0.078.

$c_{alcohol}$	$10^4 \cdot k_{obs}$	k/k_h	$(k/k_h - 1)/c_{alk}$
0.00 m	17.5	—	—
0.01 m	14.7	1.191	19.1
0.02 m	12.9	1.357	17.9
0.04 m	11.4	1.535	13.4
0.08 m	9.8	1.786	9.8
0.12 m	7.8	2.244	10.4
		average...	14.1

$$K_{m_2} = \frac{0.031 \cdot 1}{0.071 \cdot 14.1} = 0.0309$$

β. 20°. Glucoside 0.0400 m. $K_m = 0.031$. $e = 0.1502$. sal. f. = 0.078.

$c_{alcohol}$	$10^4 \cdot k_{obs}$	k/k_h	$(k/k_h - 1)/c_{alk}$
0.00 m	16.3	—	—
0.01 m	13.4	1.216	21.6
0.02 m	11.5	1.417	20.9
0.04 m	10.0	1.630	15.8
0.08 m	8.9	1.832	10.4
0.12 m	7.4	2.203	10.0
		average...	15.7

$$K_{m_2} = \frac{0.031 \cdot 1}{0.071 \cdot 15.7} = 0.0278$$

K_{m_2}, average value at 30° and 20°, 0.029.

We have now established

$$K_m = 0.031.\ K_{m_1} = 0.180.\ K_{m_2} = 0.029.\ K_m/K_{m_1} = 0.172.$$
$$K_m/K_{m_2} = 1.069.\ (K_m/K_{m_1} + K_m/K_{m_2} - 1) = 0.242.$$

i. e. it is to be expected that the observed constants will decrease slightly during the hydrolysis.

e. Examples of calculation of k_{obs} ($K_m + c + (K_m/K_{m_1} + K_m/K_{m_2} - 1)\,x) = k_3 \cdot e$ (sal. f.).

Table V.

Glucoside 0.0400 m. 30°. e = 0.1481. sal. f. = 0.044.

No toluene.

$\alpha_{beg.} = -0.600°$. $\alpha_{end} = +0.620°$. $\alpha_{emulsin} = -0.290°$.

$K_m = 0.031$. $K_{m_1} = 0.180$. $K_{m_2} = 0.029$.

$(K_m/K_{m_1} + K_m/K_{m_2} - 1) = 0.242$.

t min	α_{obs}	$\alpha_{end} - \alpha_t$	$c_{glucoside}$	x	$k \cdot 10^4$	$(K_m/K_{m_1} + K_m/K_{m_2} - 1)\,x$	$10^4 \cdot k_3 \cdot e$ (sal. f.)
0	—0.890	1.220	0.0400	0.0000	—	—	—
30	—0.730	1.060	0.0348	0.0052	20.4	0.0007	1.44
60	—0.615	0.945	0.0310	0.0090	16.6	0.0018	1.34
90	—0.505	0.835	0.0274	0.0126	17.9	0.0026	1.33
120	—0.420	0.750	0.0246	0.0154	15.5	0.0034	1.30
150	—0.330	0.660	0.0216	0.0184	18.5	0.0041	1.32
180	—0.260	0.590	0.0194	0.0206	16.2	0.0048	1.31
240	—0.125	0.455	0.0149	0.0251	18.8	0.0055	1.35
300	—0.025	0.355	0.0116	0.0284	18.0	0.0065	1.37
				average...	17.7		1.35

k_{obs}/e (sal. f.) = $27.2 \cdot 10^{-2}$. $k_3 = 2.04 \cdot 10^{-2}$.

Table VI.

Glucoside 0.0400 m. 20°. e = 0.2149. sal. f. = 0.044.

No toluene.

$\alpha_{beg} = -0.600°$. $\alpha_{end} = +0.620°$. $\alpha_{emulsin} = -0.425°$.

t min	α_{obs}	$\alpha_{end} - \alpha_t$	$c_{glucoside}$	x	$k \cdot 10^4$	$(K_m/K_{m_1} + K_m/K_{m_2} - 1)\,x$	$10^4 \cdot k_3 \cdot e$ (sal. f.)
0	—1.025	1.220	0.0400	0.0000	—	—	—
20	—0.955	1.150	0.0377	0.0023	12.8	0.0003	0.91
40	—0.890	1.085	0.0357	0.0044	12.6	0.0009	0.91
60	—0.825	1.020	0.0334	0.0066	13.4	0.0014	0.97
90	—0.740	0.935	0.0307	0.0093	12.6	0.0020	0.92
120	—0.670	0.865	0.0282	0.0118	11.3	0.0026	0.83
180	—0.530	0.725	0.0244	0.0156	12.8	0.0034	0.95
240	—0.420	0.615	0.0202	0.0198	11.9	0.0043	0.90
300	—0.330	0.525	0.0172	0.0228	11.5	0.0052	0.87
				average...	12.3		0.91

k_{obs}/e (sal. f.) = $13.1 \cdot 10^{-2}$. $k_3 = 0.96 \cdot 10^{-2}$.

Table VII.

Glucoside 0.0400 m. 30°. $c_{alcohol} = 0.0400$.

$0.04 \cdot K_m/K_{m_2} = 0.043$. No toluene.

e = 0.0763. sal. f. = 0.078.

$\alpha_{emulsin} = -0.125°$. $\alpha_{beg} = -0.600°$. $\alpha_{end} = +0.620°$.

t min	α_{obs}	$\alpha_{end} - \alpha_t$	$c_{glucoside}$	x	$k \cdot 10^4$	$(K_m/K_{m_1} + K_m/K_{m_2} - 1)\,x$	$10^4 \cdot k_3 \cdot e$ (sal. f.)
0	—0.725	1.220	0.0400	0.0000	—	—	—
20	—0.665	1.160	0.0380	0.0020	10.9	0.0003	1.25
40	—0.610	1.105	0.0362	0.0038	10.6	0.0007	1.21
60	—0.560	1.055	0.0346	0.0054	10.1	0.0011	1.16
90	—0.485	0.980	0.0321	0.0079	10.7	0.0016	1.23
120	—0.410	0.905	0.0297	0.0103	11.5	0.0022	1.34
180	—0.290	0.785	0.0257	0.0143	10.3	0.0030	1.20
240	—0.165	0.660	0.0216	0.0184	12.6	0.0040	1.48
300	—0.045	0.540	0.0177	0.0233	14.4	0.0049	1.71
				average...	11.4		1.32

k_{obs}/e (sal. f.) = $19.2 \cdot 10^{-2}$. $k_3 = 2.17 \cdot 10^{-2}$.

Table VIII.

Glucoside 0.0400 m. 30°. $c_{glucose} = 0.0400$.

$0.04 \cdot K_m/K_{m_1} = 0.007$.

1 ml toluene to 50 ml solution. e = 0.0763. sal. f. = 0.078.

$\alpha_{emulsin} = -0.120°$. $\alpha_{beg} = +0.020°$. $\alpha_{end} = +1.240°$.

t min	α_{obs}	$\alpha_{end} - \alpha_t$	$c_{glucoside}$	x	$k \cdot 10^4$	$(K_m/K_{m_1} + K_m/K_{m_2} - 1)\,x$	$10^4 \cdot k_3 \cdot e$ (sal. f.)
0	—0.100	1.220	0.0400	0.0000	—	—	—
20	+0.010	1.110	0.0364	0.0036	20.5	0.0005	1.61
40	+0.110	1.010	0.0331	0.0069	20.5	0.0013	1.62
60	+0.180	0.940	0.0308	0.0092	15.6	0.0020	1.26
90	+0.285	0.835	0.0274	0.0126	17.1	0.0026	1.38
120	+0.420	0.700	0.0230	0.0170	25.5	0.0036	2.08
180	+0.590	0.530	0.0174	0.0226	20.1	0.0048	1.67
240	+0.725	0.395	0.0128	0.0272	22.2	0.0061	1.80
				average...	20.1		1.63

k_{obs}/e (sal. f.) = $33.0 \cdot 10^{-2}$. $k_3 = 2.68 \cdot 10^{-2}$.

Table IX.

n-Butyl-β-d-glucoside. Summary of results obtained.

$c_{glucoside}$	$c_{glucose}$	$c_{alcohol}$	$10^4 \cdot k_{obs}$	e	sal. f.	$\frac{10^2 \cdot k_{obs}}{e\ (sal.\ f.)}$	$10^2 \cdot k_3$
			1. 30°. No toluene.				
0.0400	0.00	0.00	8.6	0.0741	0.044	26.4	1.93
0.0400	0.00	0.00	17.7	0.1481	0.044	27.2	2.04
0.0400	0.00	0.00	28.0	0.2222	0.044	28.7	2.18
0.0400	0.00	0.00	26.6	0.2149	0.044	28.1	2.21
0.0100	0.00	0.00	61.2	0.0920	0.078	(85.3)	2.56
0.0200	0.00	0.00	33.3	0.0920	0.078	(46.4)	2.41
0.0400	0.00	0.00	19.3	0.0920	0.078	26.9	1.94
0.0800	0.00	0.00	11.8	0.0920	0.078	(16.4)	1.84
0.1200	0.00	0.00	9.1	0.0920	0.078	(12.7)	1.92
0.1600	0.00	0.00	7.2	0.0920	0.078	(10.0)	1.88
0.0200	0.00	0.00	40.2	0.2120	0.044	(43.1)	2.25
0.0400	0.00	0.00	25.7	0.2120	0.044	27.6	2.00
0.0800	0.00	0.00	17.3	0.2120	0.044	(18.5)	2.10
0.1600	0.00	0.00	11.7	0.2120	0.044	(12.5)	2.44
0.2400	0.00	0.00	8.5	0.2120	0.044	(9.1)	2.50
0.3200	0.00	0.00	7.1	0.2120	0.044	(7.6)	2.71
0.0400	0.00	0.00	17.5	0.0763	0.078	29.4	2.10
0.0400	0.00	0.01	14.7	0.0763	0.078	(24.2)	2.04
0.0400	0.00	0.02	12.9	0.0763	0.078	(21.7)	2.00
0.0400	0.00	0.04	11.4	0.0763	0.078	(19.2)	2.17
0.0400	0.00	0.08	9.8	0.0763	0.078	(16.4)	2.55
0.0400	0.00	0.12	7.8	0.0763	0.078	(13.1)	2.55
					average...	27.8	2.20
						c = 0.0400.	
			2. 20°. No toluene.				
0.0400	0.00	0.00	12.3	0.2149	0.044	13.1	0.96
0.0200	0.00	0.00	18.1	0.1014	0.078	(22.9)	1.19
0.0400	0.00	0.00	12.6	0.1014	0.078	15.9	1.15
0.0800	0.00	0.00	7.3	0.1014	0.078	(9.2)	1.04
0.1600	0.00	0.00	4.8	0.1014	0.078	(6.1)	1.16
0.2400	0.00	0.00	3.6	0.1014	0.078	(4.6)	1.23
0.3200	0.00	0.00	3.0	0.1014	0.078	(3.8)	1.32
0.0400	0.00	0.00	16.3	0.1502	0.078	13.9	1.03
0.0400	0.00	0.01	13.4	0.1502	0.078	(11.4)	0.96
0.0400	0.00	0.02	11.5	0.1502	0.078	(9.8)	0.93

(continued)

$c_{glucoside}$	$c_{glucose}$	$c_{alcohol}$	$10^4 \cdot k_{obs}$	e	sal. f.	$\frac{10^2 \cdot k_{obs}}{e\ (sal.\ f.)}$	$10^2 \cdot k_3$
0.0400	0.00	0.04	10.0	0.1502	0.078	(8.5)	0.99
0.0400	0.00	0.08	8.9	0.1502	0.078	(7.6)	1.20
0.0400	0.00	0.12	7.4	0.1502	0.078	(6.3)	1.28
					average...	14.3	1.11

c = 0.0400.

3. 30°. 1 ml toluene to 50 ml solution.

0.0400	0.00	0.00	21.7	0.0763	0.078	35.6	2.63
0.0400	0.01	0.00	20.8	0.0763	0.078	(34.2)	2.56
0.0400	0.02	0.00	20.2	0.0763	0.078	(33.2)	2.56
0.0400	0.04	0.00	20.1	0.0763	0.078	(33.0)	2.69
0.0400	0.08	0.00	19.6	0.0763	0.078	(32.2)	2.68
0.0400	0.12	0.00	19.1	0.0763	0.078	(31.4)	2.94
					average...	35.6	2.68

c = 0.0400.

$k_{3_{30}}/k_{3_{20}} = 2.20/1.11 = 2.0$.

Effect of toluene: 2.68/2.20 = 1.22.

Effect of toluene, directly observed constants: 35.6/27.8 = 1.28.

2. iso-Butyl-β-d-glucoside.

Determination of K_m.

Table X.

a. 30°. e = 0.0740.

sal. f. = 0.078.

c	$10^4 \cdot k'_{obs}$	e (sal. f.)/k'_{obs}
0.0100	34.78	1.66
0.0200	24.63	2.34
0.0300	18.78	3.07
0.0400	15.92	3.63
0.0600	11.34	5.09
0.0800	9.23	6.25

Table XI.

b. 20°. e = 0.1504.

sal. f. = 0.078.

c	$10^4 \cdot k'_{obs}$	e (sal. f.)/k'_{obs}
0.0100	30.67	3.82
0.0200	21.06	5.57
0.0300	17.03	6.89
0.0400	13.79	8.51
0.0600	10.96	10.70
0.0800	9.35	12.55

Fig. 2 shows that the value of K_m, both at 30° and at 20°, is 0.013. In the figure are introduced experiments at

higher glucoside concentrations than those used in tables X—XI; it is seen here as in the case of n-butyl-glucoside that at higher concentrations the k'_{obs}-values are found too high.

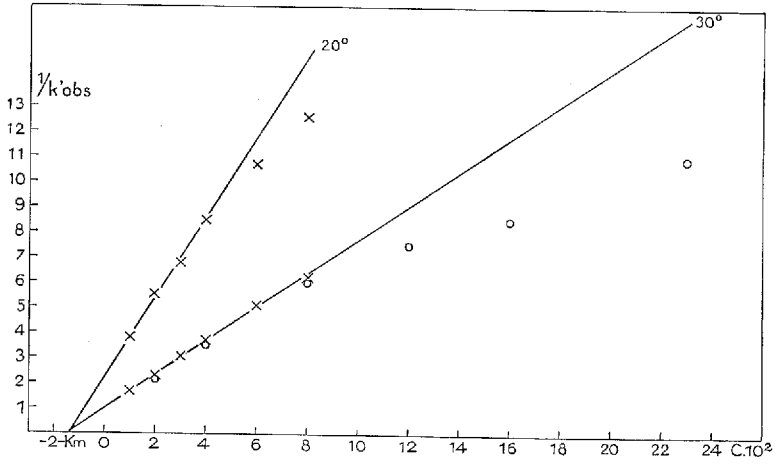

Fig. 2.

c. Determination of K_{m_1}, the affinity constant of glucose.

Table XII.

30°. Glucoside 0.0400 m. $K_m = 0.013$. $e = 0.0717$.
sal. f. = 0.078.
1 ml toluene to 50 ml solution.

$c_{glucose}$	$10^4 \cdot k_{obs}$	k/k_h	$K_{m_1} = \frac{K_m \cdot c_{glucose}}{(K_m + c)(k/k_h - 1)}$
0.00 m	16.4	—	—
0.01 m	16.1	1.019	0.129
0.02 m	15.7	1.045	0.109
0.04 m	15.1	1.086	0.114
0.08 m	14.6	1.123	0.160
0.12 m	13.3	1.233	0.126
		average...	0.13

This value is lower than the average value previously found with other glucosides, 0.18. As the value 0.18, however, coincides with the value found by other investigators, we shall regard this value as more accurate than the value found here and consequently we use $K_{m_1} = 0.18$ in the calculation of k_3 for isobutylglucoside. The present experiment is recorded only as an example of the deviations which may occur in experiments dealing with enzyme preparations.

d. Determination of K_{m_2}, the affinity constant of iso-butyl-alcohol.

Table XIII.

30°. Glucoside 0.0400 m. $K_m = 0.013$. e = 0.0635. sal. f. = 0.078.

$c_{alcohol}$	$10^4 \cdot k_{obs}$	k/k_h	$(k/k_h - 1)/c_{alk}$
0.00 m	13.3	—	—
0.01 m	12.1	1.100	10.0
0.02 m	11.3	1.180	9.0
0.04 m	10.3	1.292	7.3
0.08 m	9.1	1.462	5.8
0.12 m	7.9	1.684	5.7
		average...	7.6

$$K_{m_2} = \frac{0.013 \cdot 1}{0.053 \cdot 7.6} = 0.032.$$

For iso-butyl-β-d-glucoside we have thus found

$$K_m = 0.013.\ (K_{m_1} = 0.180).\ K_{m_2} = 0.032.\ K_m/K_{m_1} = 0.072.$$
$$K_m/K_{m_2} = 0.406.\ (K_m/K_{m_1} + K_m/K_{m_2} - 1) = -0.522,$$

i. e. it is to be expected that the observed constants will increase somewhat during the hydrolysis.

e. Examples of calculations of $k_{obs}(K_m + c + (K_m/K_{m_1} + K_m/K_{m_2} - 1)x) = k_3 \cdot e(sal.\ f.)$.

Table XIV.

Glucoside 0.0400 m. 30°. e = 0.1580. sal. f. = 0.044.

No toluene.

$\alpha_{beg} = -0.655°$. $\alpha_{end} = +0.620°$. $\alpha_{emulsin} = -0.310°$.

$K_m = 0.013$. $K_{m_1} = 0.180$. $K_{m_2} = 0.032$.

$(K_m/K_{m_1} + K_m/K_{m_2} - 1) = -0.522$.

t min	α_{obs}	$\alpha_{end} - \alpha_t$	$c_{glucose}$	x	$k \cdot 10^4$	$(K_m/K_{m_1} + K_m/K_{m_2} - 1)\,x$	$10^4 \cdot k_3 \cdot e$ (sal. f.)
0	—0.965	1.275	0.0400	0.0000	—	—	—
30	—0.805	1.115	0.0350	0.0050	19.4	—0.0013	0.96
60	—0.670	0.980	0.0308	0.0092	18.7	—0.0034	0.93
90	—0.555	0.865	0.0271	0.0129	18.1	—0.0054	0.86
120	—0.455	0.765	0.0240	0.0160	17.8	—0.0075	0.81
150	—0.355	0.665	0.0209	0.0191	20.3	—0.0092	0.89
180	—0.265	0.575	0.0180	0.0220	21.1	—0.0107	0.89
240	—0.125	0.435	0.0137	0.0263	20.2	—0.0126	0.82
300	—0.005	0.315	0.0099	0.0301	23.4	—0.0147	0.89
				average...	19.9		0.88

k_{obs}/e (sal. f.) $= 28.6 \cdot 10^{-2}$. $k_3 = 1.27 \cdot 10^{-2}$.

Table XV.

Glucoside 0.0400 m. 20°. e = 0.2128. sal. f. = 0.044.

No toluene.

$\alpha_{beg} = -0.655°$. $\alpha_{end} = +0.620°$. $\alpha_{emulsin} = -0.415$.

t min	α_{obs}	$\alpha_{end} - \alpha_t$	$c_{glucoside}$	x	$k \cdot 10^4$	$(K_m/K_{m_1} + K_m/K_{m_2} - 1)\,x$	$10^4 \cdot k_3 \cdot e$ (sal. f.)
0	—1.070	1.275	0.0400	0.0000	—	—	—
15	—1.020	1.225	0.0384	0.0016	11.6	—0.0004	0.61
30	—0.965	1.170	0.0367	0.0033	13.3	—0.0012	0.69
45	—0.915	1.120	0.0351	0.0049	12.6	—0.0021	0.64
60	—0.865	1.070	0.0336	0.0064	13.2	—0.0029	0.66
90	—0.780	0.985	0.0309	0.0091	12.0	—0.0040	0.59
120	—0.720	0.925	0.0290	0.0110	9.1	—0.0053	0.43
150	—0.650	0.855	0.0268	0.0132	11.4	—0.0064	0.53
180	—0.580	0.785	0.0246	0.0154	12.4	—0.0074	0.56
				average...	12.0		0.59

k_{obs}/e (sal. f.) $= 12.8 \cdot 10^{-2}$. $k_3 = 0.63 \cdot 10^{-2}$.

Table XVI.

Glucoside 0.0400 m. 30°. $c_{alcohol} = 0.04$ m.

$0.04 \cdot K_m/K_{m_2} = 0.016$.

No toluene. e = 0.0655. sal. f. = 0.078.

$\alpha_{beg} = -0.655°$. $\alpha_{end} = +0.620°$. $\alpha_{emulsin} = -0.105°$.

t min	α_{obs}	$\alpha_{end} - \alpha_t$	$c_{glucoside}$	x	$k \cdot 10^4$	$(K_m/K_{m_1} + K_m/K_{m_2} - 1)\,x$	$10^4 \cdot k_3 \cdot e$ (sal. f.)
0	—0.760	1.275	0.0400	0.0000	—	—	—
20	—0.705	1.220	0.0383	0.0017	9.6	—0.0004	0.66
40	—0.655	1.170	0.0367	0.0033	9.1	—0.0013	0.62
60	—0.600	1.115	0.0350	0.0050	10.5	—0.0021	0.70
90	—0.525	1.040	0.0326	0.0074	10.1	—0.0032	0.67
120	—0.465	0.980	0.0308	0.0092	8.7	—0.0043	0.56
150	—0.395	0.910	0.0285	0.0115	10.7	—0.0054	0.68
180	—0.330	0.845	0.0265	0.0135	10.7	—0.0065	0.67
240	—0.220	0.735	0.0231	0.0169	10.1	—0.0079	0.62
300	—0.100	0.615	0.0193	0.0207	12.9	—0.0098	0.77
				average...	10.3		0.66

k_{obs}/e (sal. f.) $= 20.2 \cdot 10^{-2}$. $k_3 = 1.29 \cdot 10^{-2}$.

Table XVII.

Glucoside 0.0400 m. Glucose 0.12 m. 30°.

$0.12 \cdot K_m/K_{m_1} = 0.009$. 1 ml toluene to 50 ml solution.

e = 0.0717. sal. f. = 0.078.

$\alpha_{beg} = +1.210°$. $\alpha_{end} = +2.485°$. $\alpha_{emulsin} = -0.110°$.

t min	α_{obs}	$\alpha_{end} - \alpha_t$	$c_{glucoside}$	x	$k \cdot 10^4$	$(K_m/K_{m_1} + K_m/K_{m_2} - 1)\,x$	$10^4 \cdot k_3 \cdot e$ (sal. f.)
0	+1.100	1.275	0.0400	0.0000	—	—	—
20	+1.175	1.200	0.0376	0.0024	13.2	0.0006	0.80
40	+1.230	1.145	0.0359	0.0041	10.2	0.0017	0.61
60	+1.300	1.075	0.0337	0.0063	13.7	0.0027	0.81
90	+1.390	0.985	0.0309	0.0091	12.7	0.0040	0.73
120	+1.475	0.900	0.0282	0.0118	13.1	0.0054	0.74
150	+1.560	0.815	0.0256	0.0144	14.4	0.0068	0.79
180	+1.625	0.750	0.0235	0.0165	12.0	0.0080	0.65
240	+1.780	0.595	0.0187	0.0213	16.8	0.0098	0.87
				average...	13.3		0.75

k_{obs}/e (sal. f.) $= 23.8 \cdot 10^{-2}$. $k_3 = 1.34 \cdot 10^{-2}$.

Table XVIII.

iso-Butyl-β-d-glucoside. Summary of results obtained.

$c_{glucoside}$	$c_{glucose}$	$c_{alcohol}$	$10^4 \cdot k_{obs}$	e	sal. f.	$\frac{10^2 \cdot k_{obs}}{e\ (sal.\ f.)}$	$10^2 \cdot k_3$
			1. 30°. No toluene.				
0.0100	0.00	0.00	35.8	0.0740	0.078	(62.0)	1.36
0.0200	0.00	0.00	24.9	0.0740	0.078	(43.1)	1.36
0.0300	0.00	0.00	18.0	0.0740	0.078	(31.2)	1.28
0.0400	0.00	0.00	15.1	0.0740	0.078	26.2	1.34
0.0600	0.00	0.00	10.6	0.0740	0.078	(18.4)	1.31
0.0800	0.00	0.00	8.8	0.0740	0.078	(15.2)	1.38
0.0400	0.00	0.00	13.3	0.0655	0.078	26.0	1.23
0.0400	0.00	0.01	12.1	0.0655	0.078	(23.7)	1.23
0.0400	0.00	0.02	11.3	0.0655	0.078	(22.1)	1.24
0.0400	0.00	0.04	10.3	0.0655	0.078	(20.2)	1.29
0.0400	0.00	0.08	9.1	0.0655	0.078	(17.8)	1.45
0.0400	0.00	0.12	7.9	0.0655	0.078	(15.5)	1.52
0.0400	0.00	0.00	9.2	0.0790	0.044	26.5	1.30
0.0400	0.00	0.00	19.9	0.1580	0.044	28.6	1.27
0.0400	0.00	0.00	30.9	0.2370	0.044	29.6	1.31
0.0400	0.00	0.00	26.1	0.2128	0.044	27.9	1.28
0.0400	0.00	0.00	16.3	0.0783	0.078	26.7	1.29
0.0200	0.00	0.00	27.4	0.0886	0.078	(39.6)	1.24
0.0400	0.00	0.00	14.9	0.0886	0.078	24.5	1.24
0.0800	0.00	0.00	10.6	0.0886	0.078	(15.3)	1.38
0.1600	0.00	0.00	6.4	0.0886	0.078	(9.3)	1.30
0.2400	0.00	0.00	4.8	0.0886	0.078	(6.9)	1.39
0.3200	0.00	0.00	3.6	0.0886	0.078	(5.2)	1.45
					average...	27.0	1.32
						c = 0.0400.	
			2. 20°. No toluene.				
0.0100	0.00	0.00	32.2	0.1504	0.078	(27.4)	0.61
0.0200	0.00	0.00	21.0	0.1504	0.078	(17.9)	0.57
0.0300	0.00	0.00	17.0	0.1504	0.078	(14.5)	0.60
0.0400	0.00	0.00	13.7	0.1504	0.078	11.7	0.60
0.0600	0.00	0.00	11.0	0.1504	0.078	(9.4)	0.66
0.0800	0.00	0.00	9.4	0.1504	0.078	(8.0)	0.72
0.0400	0.00	0.00	12.0	0.2128	0.044	12.8	0.63
0.0400	0.00	0.00	16.5	0.1566	0.078	13.5	0.65
					average...	12.7	0.63
						c = 0.0400.	

(continued)

$c_{glucoside}$	$c_{glucose}$	$c_{alcohol}$	$10^4 \cdot k_{obs}$	e	sal. f.	$\frac{10^2 \cdot k_{obs}}{e\ (sal.\ f.)}$	$10^2 \cdot k_3$
3. 30°. 1 ml toluene to 50 ml solution.							
0.0400	0.00	0.00	16.4	0.0717	0.078	29.3	1.39
0.0400	0.01	0.00	16.1	0.0717	0.078	(28.8)	1.36
0.0400	0.02	0.00	15.7	0.0717	0.078	(28.1)	1.36
0.0400	0.04	0.00	15.1	0.0717	0.078	(27.0)	1.36
0.0400	0.08	0.00	14.6	0.0717	0.078	(26.1)	1.39
0.0400	0.12	0.00	13.3	0.0717	0.078	(23.8)	1.34
					average...	29.3	1.37

$k_{3_{30}}/k_{3_{20}} = 1.32/0.63 = 2.1.$

Effect of toluene 1.37/1.32 = 1.04.

Effect of toluene directly observed 29.3/27.0 = 1.09.

3. l-Methyl-ethyl-carbinol-β-d-glucoside.

Determination of K_m.

Table XIX.

a. 30°. e = 0.0174.

sal. f. = 0.078.

c	$10^4 \cdot k'_{obs}$	e (sal. f.)/k'_{obs}
0.0100	46.26	0.293
0.0200	40.16	0.338
0.0400	28.32	0.479
0.0800	20.29	0.669
0.1200	15.97	0.850
0.1600	13.20	1.028

Table XX.

b. 20°. e = 0.0320.

sal. f. = 0.078.

c	$10^4 \cdot k'_{obs}$	e (sal. f.)/k'_{obs}
0.0100	45.71	0.546
0.0200	39.50	0.632
0.0400	28.92	0.863
0.0800	19.98	1.249
0.1200	16.13	1.547
0.1600	13.18	1.894

Fig. 3 shows that the value of K_m, both at 30° and at 20°, is 0.045.

c. Determination of K_{m_1}, the affinity constant of glucose. On account of lack of material we have not determined the K_{m_1}-value, but we have made use of the usual value, 0.18.

d. Determination of K_{m_2}, the affinity constant of l-methyl-ethyl-carbinol.

For the determination of K_{m_2} we have not had the optically pure l-methyl-ethyl-carbinol at our disposal, but only

the racemic carbinol and a preparation containing 72% l- and 28% d-carbinol. With these two preparations we have determined the K_{m_2}-values and thereupon by calculation the K_{m_2}-values of the l- and the d-carbinol respectively, taking it for granted that the actions of the two carbinols may be added arithmetically.

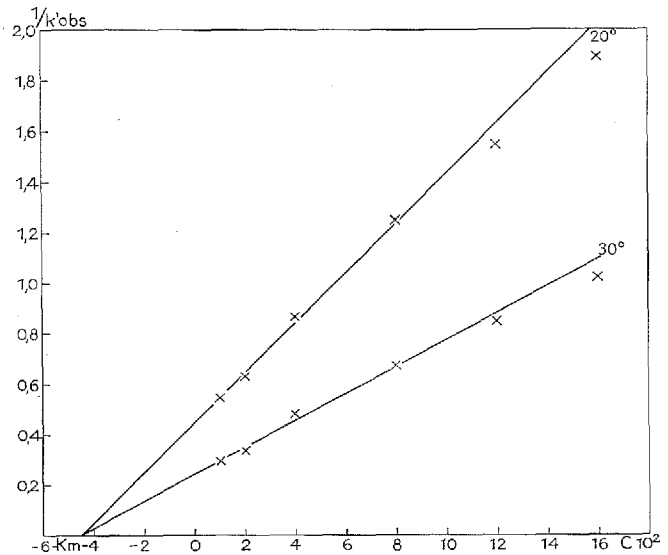

Fig. 3.

Table XXI.

α. K_{m_2} for the d, l-carbinol.

30°. Glucoside 0.0400 m. $K_m = 0.045$. e = 0.0196.

sal. f. = 0.078.

$c_{alcohol}$	$10^4 \cdot k_{obs}$	k/k_h	$K_{m_2} = \frac{K_m \cdot c_{alcohol}}{(K_m + c)(k/k_h - 1)}$
0.00	30.8	—	—
0.01	27.8	1.108	0.0490
0.02	25.8	1.194	0.0546
0.04	22.4	1.385	0.0556
0.08	18.2	1.702	0.0601
0.12	15.1	2.040	0.0611
		average...	0.0561

Table XXII.

β. K_{m_2} for the "l-carbinol" (72% l-carbinol).
30°. Glucoside 0.0400 m. $K_m = 0.045$. c = 0.0144.
sal. f. = 0.078.

$c_{alcohol}$	$10^4 \cdot k_{obs}$	k/k_h	$K_{m_2} = \frac{K_m \cdot c_{alcohol}}{(K_m + c)(k/k_h - 1)}$
0.00	24.3	—	—
0.01	20.5	1.185	0.0286
0.02	17.5	1.389	0.0272
0.04	15.6	1.558	0.0380
0.08	12.4	1.960	0.0441
0.12	10.6	2.293	0.0491
		average...	0.0374

For the calculation of K_{m_2l} and K_{m_2d} we have now the equations

$$0.50\,K_{m_2l} + 0.50\,K_{m_2d} = 0.0561$$
$$0.72\,K_{m_2l} + 0.28\,K_{m_2d} = 0.0374$$

from which we get

$$K_{m_2l} = 0.014, \qquad K_{m_2d} = 0.098,$$

i. e. the affinity of emulsin to l-methyl-ethyl-carbinol is much greater than the affinity to d-methyl-ethyl-carbinol.

For l-methyl-ethyl-carbinol-β-d-glucoside we have thus found

$$K_m = 0.045.\ (K_{m_1} = 0.180).\ K_{m_2l} = 0.014.\ K_{m_2d,l} = 0.056.$$
$$K_m/K_{m_1} = 0.250.\ K_m/K_{m_2l} = 3.214.\ K_m/K_{m_2d,l} = 0.804.$$
$$(K_m/K_{m_1} + K_m/K_{m_2l} - 1) = 2.464,$$

i. e. it is to be expected that the constants observed will show a rather great decrease during the hydrolysis.

e. Examples of the calculation of $k_{obs}(K_m + c + (K_m/K_{m_1} + K_m/K_{m_2} - 1)\,x) = k_3$.

For l-methyl-ethyl-carbinol-β-d-glucoside the end-value of rotation is different from the end-value of glucosides with an inactive aglucone. For 0.04 molar solutions of l-methyl-ethyl-carbinol the rotation, measured in 2 dm-tubes, is $-0.080°$ and the end-value for a 0.04 m glucoside-solution is consequently $+0.540°$ instead of $+0.620°$.

Table XXIII.

Glucoside 0.0400 m. 30°. e = 0.0213. sal. f. = 0.078.

No toluene.

$\alpha_{beg} = -0.730°$. $\alpha_{end} = +0.540°$. $\alpha_{emulsin} = -0.035°$.

$K_m = 0.045$. $K_{m_1} = 0.180$. $K_{m_2} = 0.014$.

$(K_m/K_{m_1} + K_m/K_{m_2} - 1) = 2.464$.

t min	α_{obs}	$\alpha_{end} - \alpha_t$	$c_{glucoside}$	x	$10^4 \cdot k$	$(K_m/K_{m_1} + K_m/K_{m_2} - 1)\,x$	$10^4 \cdot k_3 \cdot e$ (sal. f.)
0	−0.765	1.270	0.0400	0.0000	—	—	—
20	−0.575	1.080	0.0340	0.0060	35.2	0.0074	3.25
40	−0.420	0.925	0.0291	0.0109	33.6	0.0209	3.56
60	−0.275	0.780	0.0246	0.0154	37.0	0.0324	4.35
90	−0.130	0.635	0.0200	0.0200	29.8	0.0436	3.83
120	−0.005	0.510	0.0161	0.0239	31.7	0.0541	4.41
180	+0.165	0.340	0.0107	0.0293	29.3	0.0656	4.42
240	+0.265	0.240	0.0076	0.0324	25.2	0.0760	4.06
300	+0.340	0.165	0.0052	0.0348	27.1	0.0828	4.55
				average...	31.1		4.05

k_{obs}/e (sal. f.) $= 187 \cdot 10^{-2}$. $k_3 = 24.4 \cdot 10^{-2}$.

Table XXIV.

Glucoside 0.0400 m. 20°. e = 0.0426. sal. f. = 0.078.

No toluene.

$\alpha_{beg} = -0.730°$. $\alpha_{end} = +0.540°$. $\alpha_{emulsin} = -0.065$.

t min	α_{obs}	$\alpha_{end} - \alpha_t$	$c_{glucoside}$	x	$10^4 \cdot k$	$(K_m/K_{m_1} + K_m/K_{m_2} - 1)\,x$	$10^4 \cdot k_3 \cdot e$ (sal. f.)
0	—0.795	1.270	0.0400	0.0000	—	—	—
20	—0.605	1.080	0.0340	0.0060	35.2	0.0074	3.25
40	—0.450	0.925	0.0291	0.0109	33.6	0.0209	3.56
60	—0.305	0.780	0.0246	0.0154	37.0	0.0324	4.35
90	—0.160	0.635	0.0200	0.0200	29.8	0.0436	3.83
120	—0.035	0.510	0.0161	0.0239	31.7	0.0541	4.41
180	+0.135	0.340	0.0107	0.0293	29.3	0.0656	4.42
240	+0.235	0.240	0.0076	0.0324	25.2	0.0760	4.06
300	+0.310	0.165	0.0052	0.0348	27.1	0.0828	4.55
				average...	31.1		4.05

k_{obs}/e (sal. f.) $= 93.6 \cdot 10^{-2}$. $k_3 = 12.2 \cdot 10^{-2}$.

Table XXV.

Glucoside 0.0400 m. 30°. d, l-carbinol 0.0400 m.

$0.04 \cdot K_m/K_{m_2 d,l} = 0.032$. No toluene.

e = 0.0196. sal. f. = 0.078.

$\alpha_{beg} = -0.730°$. $\alpha_{end} = +0.540°$. $\alpha_{emulsin} = -0.025°$.

t min	α_{obs}	$\alpha_{end} - \alpha_t$	$c_{glucoside}$	x	$10^4 \cdot k$	$(K_m/K_{m_1} + K_m/K_{m_2} - 1)\,x$	$10^4 \cdot k_3 \cdot e$ (sal. f.)
0	—0.755	1.270	0.0400	0.0000	—	—	—
20	—0.625	1.140	0.0358	0.0042	23.4	0.0052	2.86
40	—0.500	1.015	0.0318	0.0082	25.2	0.0153	3.34
60	—0.410	0.925	0.0288	0.0112	20.1	0.0238	2.83
90	—0.285	0.800	0.0248	0.0152	21.0	0.0321	3.14
				average...	22.4		3.04

k_{obs}/e (sal. f.) $= 147 \cdot 10^{-2}$. $k_3 = 19.9 \cdot 10^{-2}$.

Table XXVI.

Glucoside 0.0400 m. 30°. "l-carbinol" (72% l) 0.04 m.

No toluene.

$0.04 \cdot K_m/K_{m_2 l} = 0.048$. e = 0.0144. sal. f. = 0.078.

$\alpha_{beg} = -0.730$. $\alpha_{end} = +0.540°$. $\alpha_{\text{"l"-carb.}} = -0.030°$.

$\alpha_{emulsin} = -0.020°$.

t min	α_{obs}	$\alpha_{end} - \alpha_t$	$c_{glucoside}$	x	$10^4 \cdot k$	$(K_m/K_{m_1} + K_m/K_{m_2} - 1)\,x$	$10^4 \cdot k_3 \cdot e$ (sal. f.)
0	—0.780	1.270	0.0400	0.0000	—	—	—
20	—0.675	1.165	0.0367	0.0033	18.7	0.0041	2.57
40	—0.600	1.095	0.0345	0.0055	13.5	0.0109	1.94
60	—0.520	1.010	0.0318	0.0082	17.5	0.0169	2.63
90	—0.435	0.925	0.0292	0.0108	12.7	0.0234	1.99
				average...	15.7		2.28

$k_{obs}/e\,(sal.\,f.) = 139 \cdot 10^{-2}$. $k_3 = 20.3 \cdot 10^{-2}$.

Table XXVII.

l-Methyl-ethyl-carbinol-β-d-glucoside. Summary of results obtained.

$c_{glucoside}$	$c_{glucose}$	$c_{alcohol}$	$10^4 \cdot k_{obs}$	e	sal. f.	$\frac{10^2 \cdot k_{obs}}{e\,(sal.\,f.)}$	$10^2 \cdot k_3$
			1. 30°. No toluene.				
0.0400	0.00	0.00	31.1	0.0213	0.078	187	24.4
0.0100	0.00	0.00	45.8	0.0174	0.078	(337)	20.8
0.0200	0.00	0.00	40.1	0.0174	0.078	(296)	22.6
0.0400	0.00	0.00	29.1	0.0174	0.078	214	21.8
0.0800	0.00	0.00	20.1	0.0174	0.078	(148)	22.2
0.1200	0.00	0.00	15.4	0.0174	0.078	(113)	22.1
0.1600	0.00	0.00	12.5	0.0174	0.078	(92)	21.8
0.0400	0.00	0.00	30.8	0.0196	0.078	202	21.9
0.0400	0.00	d, l 0.01	27.8	0.0196	0.078	(182)	21.0
0.0400	0.00	d, l 0.02	25.8	0.0196	0.078	(169)	20.5
0.0400	0.00	d, l 0.04	22.4	0.0196	0.078	(147)	19.9
0.0400	0.00	d, l 0.08	18.2	0.0196	0.088	(119)	19.8
0.0400	0.00	d, l 0.12	15.1	0.0196	0.078	(99)	19.3
0.0400	0.00	0.00	24.3	0.0144	0.078	216	22.5
0.0400	0.00	"l" 0.01	20.5	0.0144	0.078	(182)	20.8
0.0400	0.00	"l" 0.02	17.5	0.0144	0.078	(156)	19.2
0.0400	0.00	"l" 0.04	15.6	0.0144	0.078	(139)	20.3
0.0400	0.00	"l" 0.08	12.4	0.0144	0.078	(110)	21.1
0.0400	0.00	"l" 0.12	10.6	0.0144	0.078	(94)	22.6
0.0400	0.00	0.00	271	0.1363	0.078	(255)	25.5
					average...	215	21.5

c = 0.0400.

(continued)

$c_{glucoside}$	$c_{glucose}$	$c_{alcohol}$	$10^4 \cdot k_{obs}$	e	sal. f.	$\frac{10^2 \cdot k_{obs}}{e\ (sal.\ f.)}$	$10^2 \cdot k_3$
			2. 20°. No toluene.				
0.0400	0.00	0.00	31.1	0.0406	0.078	94	12.2
0.0100	0.00	0.00	45.8	0.0320	0.078	(183)	11.3
0.0200	0.00	0.00	37.2	0.0320	0.078	(149)	11.3
0.0400	0.00	0.00	29.7	0.0320	0.078	119	12.1
0.0800	0.00	0.00	19.2	0.0320	0.078	(77)	11.4
0.1200	0.00	0.00	15.0	0.0320	0.078	(60)	11.6
0.1600	0.00	0.00	12.5	0.0320	0.078	(50)	11.8
0.0400	0.00	0.00	277	0.2726	0.078	130	13.0
					average...	114	11.8
						c = 0.0400.	

$k_{3_{30}}/k_{3_{20}} = 21.5/11.8 = 1.75.$

4. d-Methyl-ethyl-carbinol-β-d-glucoside.

Determination of K_m.

Table XXVIII.

a. 30°. e = 0.0682.

sal. f. = 0.078

c	$10^4 \cdot k'_{obs}$	e (sal. f.)/k'_{obs}
0.0100	51.80	1.026
0.0200	40.94	1.300
0.0400	29.98	1.774
0.0600	23.35	2.278
0.1000	17.98	2.959
0.1600	12.44	4.276

Table XXIX.

b. 20°. e = 0.1252.

sal. f. = 0.078.

c	$10^4 \cdot k'_{obs}$	e (sal. f.)/k'_{obs}
0.0100	51.18	1.904
0.0200	38.26	2.547
0.0400	24.45	3.985
0.0600	20.23	4.816
0.1000	14.37	6.780
0.1600	11.45	8.509

Fig. 4 shows that the value of K_m, both at 30° and at 20°, is 0.030.

c. Determination of K_{m_1}, the affinity constant of glucose. Here also we have made use of the value 0.18 for K_{m_1}, i. e. the value determined in previous investigations and by other investigators.

d. Determination of K_{m_2}, the affinity constant of d-methyl-ethyl-carbinol.

As pointed out above (p. 23) we have not had at our disposal pure d- and l-methyl-ethyl-carbinol, but only the racemic carbinol and a preparation with 72% l- and 28%

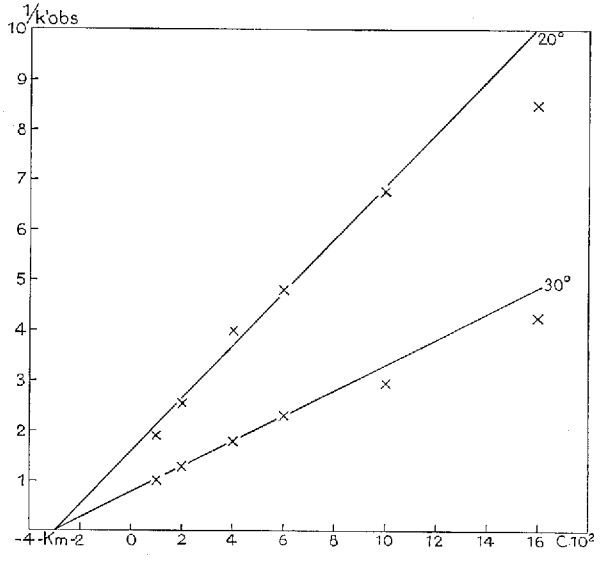

Fig. 4.

d-carbinol. Above we have calculated for the d-carbinol $K_{m_2} = 0.098$ and we have found for the d, l-carbinol $K_{m_2} = 0.056$. This value was determined with the l-methyl-ethyl-carbinol-glucoside as substrate, the K_m-value of which is 0.045. As a check on the determination of K_{m_2d} we have made a few determinations of the inhibiting effect of the d, l-carbinol on the hydrolysis of d-methyl-ethyl-carbinol-β-d-glucoside. Lack of material has rendered it impossible to carry out the determination of K_{m_2} also for the carbinol-preparation more rich in l-carbinol.

Table XXX.

K_{m_2} for the d, l-carbinol.

30°. Glucoside 0.0400 m. $K_m = 0.030$. e = 0.0292.

sal. f. = 0.078.

$c_{alcohol}$	$10^4 \cdot k_{obs}$	k/k_h	$K_{m_2} = \frac{K_m \cdot c_{alcohol}}{(K_m + c)(k/k_h - 1)}$
0.00	11.3	—	—
0.04	8.5	1.330	0.0520
0.08	7.2	1.568	0.0604
			average... 0.056

This value happens to be identical with the value 0.056 determined with the l-glucoside, and we therefore think that the calculated value of K_{m_2d} also is approximately correct.

For d-methyl-ethyl-carbinol-β-d-glucoside the following values are to be considered as the best:

$$K_m = 0.030. \ (K_{m_1} = 0.180). \ K_{m_2d} = 0.098. \ K_{m_2d,l} = 0.056.$$

$$K_m/K_{m_1} = 0.167. \ K_m/K_{m_2d} = 0.306. \ K_m/K_{m_2d,l} = 0.536.$$

$$(K_m/K_{m_1} + K_m/K_{m_2d} - 1) = -0.527,$$

i. e. it is to be expected that the constants will increase with increasing hydrolysis, but the determinations are not so sure in this case as in the cases examined before.

e. Examples of calculation of $k_{obs}(K_m + c + (K_m/K_{m_1} + K_m/K_{m_2} - 1)\,x) = k_3$.

As pointed out above (p. 26) the end-value of rotation of a glucoside with an active aglucone is different from that of a glucoside with an inactive or racemic aglucone. For 0.0400 m solutions of d-methyl-ethyl-carbinol-β-d-glucoside the end value is +0.700° (2 dm tube).

Table XXXI.

Glucoside 0.0400 m. 30°. e = 0.0930. sal. f. = 0.078.

No toluene.

$\alpha_{beg} = -0.525°$. $\alpha_{end} = +0.700°$. $\alpha_{emulsin} = -0.140°$.

$K_m = 0.030$. $K_{m_1} = 0.180$. $K_{m_2} = 0.098$.

$(K_m/K_{m_1} + K_m/K_{m_2} - 1) = -0.527$.

t min	α_{obs}	$\alpha_{end} - \alpha_t$	$c_{glucoside}$	x	$10^4 \cdot k$	$(K_m/K_{m_1} + K_m/K_{m_2} - 1)\,x$	$10^4 \cdot k_3 \cdot e$ (sal. f.)
0	—0.665	1.225	0.0400	0.0000	—	—	—
10	—0.555	1.115	0.0364	0.0036	40.9	—0.0009	2.82
20	—0.465	1.025	0.0335	0.0065	36.6	—0.0026	2.46
40	—0.310	0.870	0.0284	0.0116	35.6	—0.0047	2.32
60	—0.185	0.745	0.0243	0.0157	33.7	—0.0072	2.12
90	—0.030	0.590	0.0193	0.0207	33.8	—0.0096	2.04
120	+0.095	0.465	0.0152	0.0248	33.7	—0.0120	2.00
				average...	35.7		2.29

k_{obs}/e (sal. f.) = $49.2 \cdot 10^{-2}$. $k_3 = 3.16 \cdot 10^{-2}$.

Table XXXII.

Glucoside 0.0400 m. 20°. e = 0.1859. sal. f. = 0.078.

No toluene.

$\alpha_{beg} = -0.525°$. $\alpha_{end} = +0.700°$. $\alpha_{emulsin} = -0.280°$.

t min	α_{obs}	$\alpha_{end} - \alpha_t$	$c_{glucoside}$	x	$10^4 \cdot k$	$(K_m/K_{m_1} + K_m/K_{m_2} - 1)\,x$	$10^4 \cdot k_3 \cdot e$ (sal. f.)
0	—0.805	1.225	0.0400	0.0000	—	—	—
10	—0.695	1.115	0.0364	0.0036	40.9	—0.0009	2.82
20	—0.600	1.020	0.0333	0.0067	38.7	—0.0027	2.60
40	—0.445	0.865	0.0282	0.0118	35.8	—0.0048	2.33
60	—0.330	0.750	0.0245	0.0155	31.0	—0.0072	1.95
90	—0.185	0.605	0.0198	0.0202	31.1	—0.0094	1.89
120	—0.065	0.485	0.0158	0.0242	32.0	—0.0117	1.87
				average...	34.9		2.24

k_{obs}/e (sal. f.) = $24.1 \cdot 10^{-2}$. $k_3 = 1.54 \cdot 10^{-2}$.

Table XXXIII.

Glucoside 0.0400 m. 30°. d, l-carbinol 0.04 m.

$0.04 \cdot K_m/K_{m_2 d,l} = 0.0214$. No toluene.

e = 0.0292. sal. f. = 0.078.

$\alpha_{beg} = -0.525°$. $\alpha_{end} = +0.700°$. $\alpha_{emulsin} = -0.045°$.

t min	α_{obs}	$\alpha_{end} - \alpha_t$	$c_{glucoside}$	x	$10^4 \cdot k$	$(K_m/K_{m_1} + K_m/K_{m_2} - 1)\,x$	$10^4 \cdot k_3 \cdot e$ (sal. f.)
0	—0.570	1.225	0.0400	0.0000	—	—	—
30	—0.495	1.150	0.0376	0.0024	9.2	—0.0006	0.83
60	—0.430	1.085	0.0354	0.0046	8.4	—0.0018	0.75
90	—0.370	1.025	0.0335	0.0065	8.2	—0.0029	0.73
120	—0.315	0.970	0.0317	0.0083	8.0	—0.0039	0.70
				average...	8.5		0.75

k_{obs}/e (sal. f.) $= 37.3 \cdot 10^{-2}$. $k_3 = 3.30 \cdot 10^{-2}$.

Table XXXIV.

d-Methyl-ethyl-carbinol-β-d-glucoside. Summary of results obtained.

$c_{glucoside}$	$c_{glucose}$	$c_{alcohol}$	$10^4 \cdot k_{obs}$	e	sal. f.	$\frac{10^2 \cdot k_{obs}}{e\ (sal.\ f.)}$	$10^2 \cdot k_3$
			1. 30°. No toluene.				
0.0400	0.00	0.00	35.7	0.0930	0.078	49.2	3.16
0.0400	0.00	0.00	11.3	0.0292	0.078	49.4	3.18
0.0400	0.00	d,l 0.04	8.5	0.0292	0.078	(38.1)	3.30
0.0400	0.00	d,l 0.08	7.2	0.0292	0.078	(32.3)	3.49
0.0100	0.00	0.00	53.8	0.0682	0.078	(101)	3.89
0.0200	0.00	0.00	36.8	0.0682	0.078	(87.1)	3.31
0.0400	0.00	0.00	28.6	0.0682	0.078	53.7	3.58
0.0600	0.00	0.00	20.8	0.0682	0.078	(39.1)	3.36
0.1000	0.00	0.00	15.6	0.0682	0.078	(29.3)	3.67
0.1600	0.00	0.00	11.4	0.0682	0.078	(21.4)	3.95
					average...	50.8	3.49

c = 0.0400.

(continued)

$c_{glucoside}$	$c_{glucose}$	$c_{alcohol}$	$10^4 \cdot k_{obs}$	e	sal. f.	$\frac{10^2 \cdot k_{obs}}{e\ (sal.\ f.)}$	$10^2 \cdot k_3$
2. 20°. No toluene.							
0.0400	0.00	0.00	34.9	0.1859	0.078	24.1	1.54
0.0100	0.00	0.00	51.2	0.1252	0.078	(52.4)	2.02
0.0200	0.00	0.00	35.8	0.1252	0.078	(36.7)	1.75
0.0400	0.00	0.00	22.7	0.1252	0.078	23.2	1.56
0.0600	0.00	0.00	18.8	0.1253	0.078	(19.3)	1.66
0.1000	0.00	0.00	13.9	0.1252	0.078	(15.6)	1.78
0.1600	0.00	0.00	10.8	0.1252	0.078	(11.1)	2.03
					average...	23.7	1.76
						c = 0.0400.	

$k3_{30}/k3_{20} = 3.49/1.76 = 1.98$.

5. Trimethylcarbinol-β-d-glucoside.

Veibel and Nielsen (10) have reported this glucoside to be nearly resistent towards the action of emulsin. Later Veibel (5) has examined its enzymic hydrolysis, making use of a more active enzyme preparation than that used by Veibel and Nielsen. The constants of velocity were referred to the amount of emulsin bound to the glucoside in the way indicated by Veibel and Eriksen (1). We have now recalculated the experiments of Veibel in the way indicated in the present paper, making use of the K_m- and K_{m_2}-values previously determined (5). The difference between the constants calculated in the two different ways is only some 2—3%, which means that the more convenient method employed in the present paper is as accurate as the more complicated method previously used.

The following affinity constants were found:

$K_m = 1.46$. $(K_{m_1} = 0.18)$. $K_{m_2} = 0.37$. $K_m/K_{m_1} = 8.110$.
$K_m/K_{m_2} = 3.946$. $(K_m/K_{m_1} + K_m/K_{m_2} - 1) = 11.06$.

Examples of calculation of $k_{obs}((K_m/K_{m_1} + K_m/K_{m_2} - 1)x + K_m + c) = k_3 \cdot e\,(sal.\ f.)$.

Table XXXV.

Glucoside 0.0400 m. e = 0.2978. sal. f. = 0.34.

1 ml toluene to 50 ml solution.

t min	α_{obs}	$\alpha_{end} - \alpha_t$	$c_{glucoside}$	x	$10^4 \cdot k_{obs}$	$(K_m/K_{m_1} + K_m/K_{m_2} - 1)x$	$10^4 \cdot k_3 \cdot e\,(sal.\ f.)$
0	—0.365	0.470	0.0400	0.0000	—	—	—
1440	—0.325	0.430	0.0366	0.0034	0.27	0.0370	0.42
2880	—0.285	0.390	0.0322	0.0068	0.29	0.0735	0.46
4320	—0.255	0.360	0.0306	0.0094	0.24	0.1039	0.39
5760	—0.230	0.335	0.2085	0.0115	0.22	0.1272	0.36
				average...	0.26		0.41

$k_{obs}/e\,(sal.\ f.) = 0.03 \cdot 10^{-2}$. $k_3 = 0.04 \cdot 10^{-2}$.

For $10^4 \cdot k_3 \cdot e\,(sal.\ f.)$ we now find 0.41 and have previously calculated 0.42.

Table XXXVI.

Glucoside 0.0800 m. 30°. Carbinol 0.0400 m.

$0.04 \cdot 3.946 = 0.1578$. 1 ml toluene to 50 ml solution.

e = 0.1780. sal. f. = 0.34.

t min	α_{obs}	$\alpha_{end} - \alpha_t$	$c_{glucoside}$	x	$10^4 \cdot k$	$(K_m/K_{m_1} + K_m/K_{m_2} - 1)x$	$10^4 \cdot k_3 \cdot e\,(sal.\ f.)$
0	—0.885	1.880	0.0800	0.0000	—	—	—
1440	—0.770	1.765	0.0751	0.0049	0.19	0.0540	0.33
2880	—0.665	1.660	0.0707	0.0093	0.18	0.1028	0.33
4320	—0.575	1.570	0.0668	0.0132	0.17	0.1460	0.31
5760	—0.485	1.480	0.0630	0.0170	0.18	0.1880	0.33
				average...	0.18		0.33

$k_{obs}/e\,(sal.\ f.) = 0.03 \cdot 10^{-2}$. $k_3 = 0.055 \cdot 10^{-2}$.

For $10^4 \cdot k_3 \cdot e\,(sal.\ f.)$ we now find 0.33 against 0.34 previously calculated.

Table XXXVII.

Trimethylcarbinol-β-d-glucoside. Summary of results obtained.

$c_{glucoside}$	$c_{glucose}$	$c_{alcohol}$	$10^4 \cdot k_{obs}$	e	sal. f.	$\frac{10^2 \cdot k_{obs}}{e\ (sal.\ f.)}$	$10^2 \cdot k_3$
		30°. 1 ml toluene to 50 ml solution.					
0.0200	0.00	0.00	0.27	0.2978	0.34	(0.027)	0.040
0.0400	0.00	0.00	0.26	0.2978	0.34	0.026	0.040
0.0800	0.00	0.00	0.25	0.2978	0.34	(0.025)	0.042
0.1600	0.00	0.00	0.25	0.2978	0.34	(0.025)	0.046
0.2400	0.00	0.00	0.23	0.2978	0.34	(0.023)	0.046
0.3200	0.00	0.00	0.21	0.2978	0.34	(0.021)	0.044
0.0800	0.00	0.00	0.195	0.1780	0.34	(0.032)	0.054
0.0800	0.00	0.02	0.190	0.1780	0.34	(0.031)	0.054
0.0800	0.00	0.04	0.180	0.1780	0.34	(0.030)	0.055
0.0800	0.00	0.08	0.169	0.1780	0.34	(0.028)	0.056
0.0800	0.00	0.16	0.160	0.1770	0.34	(0.026)	0.059
0.0800	0.00	0.24	0.153	0.1780	0.34	(0.025)	0.064
						average...	0.05

The heat of activation has not been determined, as the hydrolysis at 20° is too slow to allow of a determination.

Table XXXVIII.

Alcohol	$\frac{10^2 \cdot k_{obs}}{e\ (sal.\ f.)}$ c = 0.0400		K_m	K_{m_1}	K_{m_2}	$\frac{K_m}{K_{m_1}} + \frac{K_m}{K_{m_2}} - 1$	$10^2 \cdot k_3$		$\frac{k_{3_{30}}}{k_{3_{20}}}$	Q	$10^2 \cdot k_3$ Toluene	k_{3_T}/k_3
	30°	20°					30°	20°				
Methyl	2.7	1.35	0.62	0.20	(∞)	2.44	1.89	0.94	2.0	12200	2.93	1.55
Ethyl	5.3	2.2	0.25	0.17	(∞)	0.39	1.58	0.77	2.0_5	12700	2.28	1.44
n-Propyl	22.6	11.3	0.16	0.21	0.18	0.78	5.08	2.34	2.1_5	13500	6.47	1.27
iso-Propyl	16.9	7.9	0.40	0.15	0.41	2.20	7.51	3.62	2.1	13100	8.13	1.08
n-Butyl	27.8	14.3	0.031	0.19	0.029	0.24	2.20	1.11	2.0	12200	2.68	1.22
iso-Butyl	27.0	12.7	0.013	0.13	0.032	—0.52	1.32	0.63	2.1	13100	1.37	1.04
l-Methyl-ethyl-carb.	215	114	0.045	—	0.014	2.46	21.5	11.8	1.8_5	10800	—	—
d-Methyl-ethyl-carb.	50.8	23.7	0.030	—	0.098	—0.53	3.49	1.76	1.9_8	12000	—	—
Trimethyl-carbinol	0.03	—	1.46	—	0.37	11.06	(0.05)	—	—	—	—	—
			average	0.18								

Discussion.

In order to facilitate the discussion of the results obtained we have in table XXXVIII given a summary of the constants determined for all glucosides examined in this series of papers.

If we look first at the directly observed velocity constants, the most striking feature is that there does not seem to be any relation between the velocity constant and the structure of aglucone. A comparison of propyl- and iso-propylglucoside seems to indicate that a glucoside of a primary alcohol is hydrolysed more easily than a glucoside of a secondary one, and the very low velocity of hydrolysis of trimethylcarbinol-glucoside might then be taken as an indication of the significance of a branching of the alkyl-chain: the more branched, the less hydrolysable. But for the butyl-glucosides this point of view cannot be maintained, as both d- and l-methyl-ethyl-carbinol-glucoside are hydrolysed with a greater velocity than the glucosides of the two primary butyl-alcohols. The butyl-glucosides present a striking example of the significance of the structure of the aglucone: l-methyl-ethyl-carbinol-glucoside is hydrolysed 7000 times as fast as the trimethylcarbinol-glucosides, at all events for 0.0400 m solutions.

The molecular weight of the aglucone has possibly a significance. The series of glucosides of primary alcohols seems to indicate that with increasing molecular weight the velocity constant increases, and the glucosides of the secondary alcohols examined agree with this rule. But it is very striking that the spatial arrangement of the atoms is more significant than is the molecular weight, the difference between l- and d-methyl-ethyl-carbinol-glucoside being

greater than between n-propyl and n-butyl-glucoside. And from the preliminary results of an examination of some glucosides of tertiary alcohols, reported by VEIBEL and LILLELUND (11) it is seen that the velocity constants are not always increasing with increasing molecular weight; this may be true for the tertiary butyl-, amyl- and hexyl-glucoside, but the tertiary heptyl-glucoside triethyl-carbinol-β-d-glucoside is hydrolysed considerably more slowly than its lower homologue, methyl-diethyl-carbinol-β-d-glucoside.

But as previously pointed out (1), we do not attach very much importance to the directly found velocity constants. The affinity between emulsin and the different substrates may vary so much that when the velocity constants are referred to emulsin combined with the substrate, the order of the constants is quite another than when referred to emulsin present in the solution. A comparison of the values of k_{obs} and k_3 proves this to be true, most strikingly perhaps for methyl-glucoside and isobutyl-glucoside, but the two propyl-glucosides, as well, clearly show the significance of the affinity between enzyme and substrate.

It is seen that the variation in affinity is very great, the dissociation constants of the enzyme-substrate-compound (K_m) varying from 1.46 to 0.013. No regularity in the relation between the structure of aglucone and the value of the affinity constant can be traced from the experiments reported here, whereas a regularity in the k_3-values may possibly be found: the 3 glucosides of secondary alcohols have all of them rather high k_3-values, the 5 glucosides of primary alcohols have values which are slightly lower (exception propyl-glucoside) and the k_3-value of the tertiary alcohol is extraordinarily low. The relation between the observed values of the velocity constants for l-methyl-ethyl-carbinol-

glucoside and trimethyl-carbinol-glucoside is 7000, whereas the relation between the corresponding k_3-values is only 4000. The material is, however, not large enough to allow of any generalisation.

The influence of the spatial arrangement, for k_{obs} as well as for K_m and k_3, is seen very clearly from the experiments with l- and d-methyl-ethyl-carbinol-glucoside. Mitchell and Macarthur (12) have proposed to determine the velocity constants for the hydrolysis of glucosides of l- and d-carbinols by applying exponential analysis to the experimental figures from hydrolysis-experiments of the glucoside of the racemic carbinol with emulsin. For the glucoside of secondary butyl alcohol they find $k_l/k_d = 5.5$, but just as it has been pointed out for another of their glucosides (Veibel (13)) it has not been examined whether the glucoside in question is really that of the racemic carbinol or whether it has been, at all events partially, resolved during the purification of the glucoside-tetracetate or the glucoside itself.

We find for the pure glucosides $k_{obs_l}/k_{obs_d} = 4.2$, but as the affinity between emulsin and the l-glucoside is not as great as that of the d-glucoside ($K_{m_l}/K_{m_d} = 1.5$) we find by calculation $k_{3_l}/k_{3_d} = 6.2$.

The affinity between emulsin and glucose has been determined in 6 cases, and the mean value of these determinations agrees with the generally adopted value 0.18. It must be admitted, though, that the deviations from the mean value are rather large. But it must also be taken into consideration that an inaccuracy in the determination of the K_m-value involves a corresponding inaccuracy in the determination of K_{m_1} and K_{m_2}.

The values of K_{m_2} prove that the affinity between emulsin

and the different alcohols may be considerable and that it may be greater than the affinity between emulsin and the glucoside. As in the case of the glucosides it seems impossible to find any regularity in the relation between the structure of the alcohol and the affinity between alcohol and emulsin.

The inhibiting action of glucose and the various alcohols may be calculated when the three K_m-values are known, provided that the inhibition is a competitive one. In the different examples of calculation of k_3 it may be seen that generally the influence of the products of hydrolysis may be corrected for by calculation, and this means that the inhibition is competitive. In some of the examples we have not succeeded in calculating really constant k_3-values, but we are of opinion that this is not due to a fundamental incorrectness of the calculation but to peculiarities in the different experiments, causing a partial inactivation of emulsin in the experiments where the calculation does not yield constant values of k_3.

We are inclined to find a proof of the correctness of our point of view in the fact that the determination of K_m, K_{m_1} and K_{m_2} allows us to predict whether the directly observed velocity constants may be expected to decrease, to be constant or to increase during the hydrolysis. A decrease of the constant might be due to an inactivation of the enzyme during its action and does not say anything about the correctness of the calculation, but increasing constants can not be explained in that way and we see no other simple explanation than the one which we have given above. Decreasing constants are most commonly met with, but we have found a striking case of a glucoside with increasing constants in isobutyl-glucoside, and the increase has the value calculated from the determined K_m-values. This

glucoside is, therefore, a very good confirmation of the correctness of the calculation proposed by us.

From the K_m-values of d-methyl-ethyl-carbinol-glucoside increasing values of the velocity constant might be expected for this glucoside also, but as seen in tables XXXI—XXXIII we find, on the contrary, decreasing constants. This is surely due to the incorrectness of the determination of K_{m_2d}. For the l-glucoside the K_m-values predict a very great decrease in the constants, and in the experiments we find constants which decrease, but not quite so much as expected from the calculation. As pointed out above (p. 23), the determination of K_{m_2d} and K_{m_2l} is not as reliable as the determinations of the other K_{m_2}-values and we are inclined, therefore, to give more credit to the agreement in the case of isobutyl-glucoside than to the non-agreement in the case of d-methyl-ethyl-carbinol-glucoside.

In order to get an impression of the correctness of the predictions as to the decrease or increase of the constants during the hydrolysis we have for all 9 glucosides examined up to the present calculated the decrease or the increase to be expected after 50 % hydrolysis, and in table XXXIX we compare the calculated figures with the experimental ones as recorded in this and the previous (1) paper.

It is seen that the only case which is completely wrong is the d-methyl-ethyl-carbinol-glucoside where, as pointed out, the K_{m_2}-determination is not as reliable as for the other alcohols. The calculated and the observed figures in table XXXIX do not quite agree, but as the observed figures are necessarily single determinations of the constants, the experimental error is very considerable and the difference between calculated and observed values is not greater than might be expected under these circumstances.

Table XXXIX.

Glucoside	$K_m + c$ $c = 0.04$	$(K_m/K_{m_1} + K_m/K_{m_2} - 1)\,x$ for $x = 0.0200$	Decrease (—) or increase (+)	
			calc %	found %
Methyl	0.66	0.049	— 7	—20
Ethyl	0.29	0.008	— 3	—25
Propyl	0.20	0.016	— 7	— 5
Isopropyl	0.44	0.044	— 9	—25
n-Butyl	0.071	0.0048	— 6	— 7
iso-Butyl	0.053	—0.010	+25	+10
l-Methyl-ethyl-carb.	0.085	0.049	—37	—15
d-Methyl-ethyl-carb.	0.070	—0.013	+15	—20
Trimethyl-carbinol	1.50	0.221	—12 (25 % hydr. only).	— 5

In a previous paper VEIBEL and LILLELUND (2) have shown that the standardisation of a β-glucosidase-preparation may be carried out not only with salicin as the substrate but with any other β-glucoside, provided the value of $k \cdot c/X \cdot e = k_3 \cdot (\text{sal. f.})$ is known, this expression being the velocity constant for 1 molar glucoside solutions, when 1 g of emulsin of enzymic force (sal. f.) 1, combined with the substrate, is present in a 50 ml solution. By means of proportionality-factors which are to be determined for each glucoside the k_3-value may be transformed into the usual standard indications of enzymic force, sal. f. and β-glucosidase-value, or into a new standard proposed by VEIBEL and LILLELUND, the salicin-value, which is $10^2 \cdot k_{obs}\,(K_m + c + (K_m/K_{m_1} + K_m/K_{m_2} - 1)\,x)/e$ (or with approximation $10^2 \cdot k_{obs}\,(K_m + c)/e$), salicin being the substrate. In the above-mentioned paper the proportionality-factors for salicin and for the first 4 of the alkyl-glucosides considered here are determined, and in table XL these proportionality-factors and the corresponding factors for the other 5 alkyl-glucosides examined are collected.

Table XL.

Aglucon	$k_3 \cdot 10^2$	Proportionality-factor for recalculation of $10^2 \cdot k_{obs}(K_m + c + (K_m/K_{m_1} + K_m/K_{m_2} - 1)x)/e$ into	
		Salicin value	sal. f.
Salicin	18.9	1.00	0.053
Methylalcohol	1.89	10.0	0.53
Ethylalcohol	1.58	11.9	0.63
Propylalcohol	5.08	3.72	0.20
iso-Propylalcohol	7.51	2.52	0.13
n-Butylalcohol	2.25	8.40	0.45
iso-Butylalcohol	1.32	14.3	0.76
l-Methyl-ethyl-carb.	21.5	0.88	0.047
d-Methyl-ethyl-carb.	3.49	5.42	0.29
Trimethylcarbinol	0.05	378	20

Most of these glucosides will have very little chance of being commonly used as standards instead of salicin, but e. g. n-butyl-β-d-glucoside presents some properties which should render it preferable as a test-substance. It has not the inconvenience of salicin, mentioned by Veibel and Lillelund (2), that the samples withdrawn and stopped by the addition of potassium carbonate become dark coloured, its preparation is not difficult and may even be carried out as a biochemical synthesis as indicated by Helferich and Lampert (14). As seen from table XXXIX K_m, K_{m_1} and K_{m_2} have such values that the constants of hydrolysis are nearly constant all through the experiment, the decrease for a 0.0400 m glucoside solution being only 6% after 50% hydrolysis and 9% when 75% have been hydrolysed. For Salicin Veibel and Lillelund (2) indicate $K_m = 0.014$, $K_{m_2} = 0.011$, and from these values it is calculated that the constants for a 0.0400 m solution after 50% hydrolysis have decreased 11% and after 75% hydrolysis 16%; for a 0.139 m solution, as indicated in the standardisation-

prescription of WEIDENHAGEN (8), the corresponding figures are 14% and 20%, i. e. the decrease for n-butyl-glucoside is only half of the decrease for salicin, and the k_3-value may without serious inexactitude be calculated by means of the simple expression [4]: $k_3 = k_{obs}(K_m + c)/e$, and the salicin-value, respectively the sal. f., of a β-glucosidase-preparation, determined by means of n-butyl-glucoside, is consequently

$$8.40 \cdot 10^2 \cdot k_{obs}(K_m + c)/e \text{ resp. } 0.45 \cdot 10^2 \cdot k_{obs}(K_m + c)/e,$$

at all events if, in the calculation of k_{obs}, only degrees of hydrolysis not greater than 50% are considered.

Summary.

The enzymic hydrolysis of all 5 butyl-β-d-glucosides has been examined and the velocity constants as well as the affinity constants for the glucosides and the products of hydrolysis have been determined. Table XXXVIII on p. 37 gives a summary of the results obtained.

It has been shown that for these glucosides also the values of the velocity constants, when referred to the amount of emulsin combined with the substrate, and when the inhibiting effect of the products of hydrolysis is considered, are to a very large extent independent of the concentration of the substrate, inhibiting substances end emulsin.

A graphical determination of K_m has been indicated.

A more convenient method for the calculation of k_3, the velocity constant referred to emulsin combined with he substrate, has been developed:

$$k_3 = k_{obs}(K_m + c + (K_m/K_{m_1} + K_m/K_{m_2} - 1)\,x)/e \text{ (sal. f.)},$$

where k_{obs} is the directly calculated velocity constant (calculated from point to point), K_m, K_{m_1} and K_{m_2} are the affinity constants of glucoside, glucose and aglucone respectively, c is the initial glucoside concentration, x the mean value of the concentration of the products of hydrolysis at the two points between which k_{obs} is calculated, e the weight in g of emulsin present in 50 ml solution and sal. f. the enzymic force of the emulsin preparation.

It has been shown that while the velocity constants of hydrolysis as a rule may be expected to decrease with increasing hydrolysis, increasing constants may also be expected, namely in cases where $(K_m/K_{m_1}+K_m/K_{m_2})$ is smaller than 1. Such a case has been found in isobutyl-β-d-glucoside.

Proportionality factors allowing the use of the butyl-glucosides as test-substances in the standardisation of β-glucosidase-preparations are given and the advantages of the application of n-butyl-β-d-glucoside as a standard substrate instead of salicin are discussed.

Thanks are due to the Carlsberg Foundation for a grant which enabled one of us (H. L.) to take part in this work.

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Indleveret til Selskabet den 31. Maj 1939.
Færdig fra Trykkeriet den 24. Februar 1940.