ENZYMIC HYDROLYSIS OF GLUCOSIDES

II. HYDROLYSIS OF PROPYL- AND ISOPROPYL-β-d-GLUCOSIDES AND SOME CONSIDERATIONS ON THE MECHANISM OF THE ENZYMIC HYDROLYSIS OF β-GLUCOSIDES

BY

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In a previous paper (Veibel and Eriksen, 1936, 2) we examined the enzymic hydrolysis of methyl- and ethyl-β-d-glucosides. We determined the hydrolysis constants and the affinity constants at 30° and at 20° as well as the inhibiting effect of the products of hydrolysis,

In the present paper the hydrolysis of propyl- and isopropyl-β-d-glucosides is examined in the same way, and the corresponding constants are determined. Below we are presenting the experimental material calculated in the same way as we have used in the previous paper in order to allow a comparison of the hydrolysis of the four glucosides examined.

This comparison shows that both the affinity constants and the hydrolysis constants differ considerably for the four glucosides, but that no proportionality exists between affinity constant and hydrolysis constant, the latter having been referred to unity of emulsin in the solution.

As the affinity constants are not the same for the different glucosides it would perhaps be more correct to refer the hydrolysis constants not to the amount of emulsin present in the solution, but to the amount of emulsin bound to the glucoside. We have recalculated the hydrolysis constants on this assumption and have found that, for each glucoside, the hydrolysis constant is inversely proportional to the concentration of the glucoside, at all events at the beginning of the hydrolysis. As pointed out in the previous paper (l. c. p. 15) the decrease in the reaction constants towards the end of the reactions may be ascribed to the inhibiting

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action of the products of hydrolysis, attached to part of the emulsin, thus diminishing the quantity bound to the glucoside. In order to take this fact into account we have recalculated all the hydrolysis constants, this time by calculating them "from point to point", and for each point we have calculated the amount of emulsin bound to the glucoside, considering the amount of inhibiting substances present. The product $k \cdot c$ should then be constant all through the hydrolysis. This is surely not the case, but the decrease of this product is not so great as the decrease of the hydrolysis constants, calculated as previously. We are therefore of opinion that the reaction which determines the velocity of hydrolysis, is the decomposition of the addition compound of glucoside and emulsin into glucose, alcohol, and emulsin.

Variation of the aglucone bound to the glucose molecule will therefore influence the velocity of hydrolysis in two ways:

a) The affinity constant, which determines the concentration of the addition-compound glucoside-emulsin, is, for a series of glucosides, dependent on the nature of the aglucone.

b) The value of k, the hydrolysis constant of the glucoside-emulsin-compound, may differ with the aglucone introduced in the glucose molecule.

If the assumptions made here are correct, it is possible to calculate the time necessary to produce any degree of hydrolysis for any concentration of the glucoside, when the following constants are known:

k_m, k_{m1}, and k_{m2}, the dissociation constants for the compounds of emulsin with the glucoside and with the two products of hydrolysis,

e and sal. f., the weight of emulsin present in 50 ml. of the solution and the enzymic power of the emulsin preparation used,

k_{obs}, the hydrolysis constant determined for one concentration of the glucoside,

$k_{obs} \cdot c / x \cdot e \cdot (sal. f.)$ then being a constant for each glucoside, x being the amount of emulsin actually bound to the glucoside, when regard is paid to the products of hydrolysis. (x is expressed as a fraction of e).

Experimental.

1. Propyl-β-d-glucoside.

The preparation of propyl-β-d-glucoside used here had M. P. 102—03° and $[\alpha]_D^{20} = -39.5°$ (VEIBEL and ERIKSEN, 1936, 1).

a) Determination of the velocity constant at 30° and its temperature coefficient.

The experiments were carried out as described previously (l. c. 1936, 2). k is calculated with the minute as unit of time and logarithms to base 10.

Table I.

Hydrolysis at 30°.

$c_{emulsin}$ 0.2188 g in 50 ml. $\alpha_{emulsin} = -0.425°$. Sal. f. $= 0.044$.

$c_{glucoside}$ 0.0400 M. $\alpha_{beg.} = -0.600°$, $\alpha_{end} = +0.620°$.

Time min.	Samples kept h.	α	corr.	α corr.	x	c — x	$k \cdot 10^4$
0	—	— 1.025	—	— 1.025	0.105	1.220	—
20	5.0	— 0.920	—	— 0.920	0.105	1.115	19.55
40	5.5	— 0.810	—	— 0.810	0.215	1.005	21.55
60	6.0	— 0.710	0.005	— 0.705	0.320	0.900	22.02
90	6.0	— 0.570	0.005	— 0.565	0.460	0.760	22.84
120	6.0	— 0.470	0.005	— 0.465	0.560	0.660	22.24
180	21.0	— 0.310	0.015	— 0.295	0.730	0.490	22.01
240	20.5	— 0.180	0.020	— 0.160	0.865	0.355	22.34

mean value... 21.8

$k/e \cdot (sal. f.) = 22.6 \cdot 10^{-2}$.

Table II.

Hydrolysis at 20°.

$c_{emulsin}$ and $c_{glucoside}$ as in Table I.

Time min.	Samples kept h.	α	corr.	α corr.	x	c — x	$k \cdot 10^4$
0	—	— 1.025	—	— 1.025	—	1.220	—
20	4.5	— 0.960	—	— 0.960	0.065	1.155	11.89
40	5.0	— 0.905	—	— 0.905	0.120	1.100	11.24
60	5.0	— 0.855	—	— 0.855	0.170	1.050	10.86
90	5.5	— 0.785	—	— 0.785	0.240	0.980	10.57
120	5.5	— 0.700	—	— 0.700	0.325	0.895	10.98
180	20.5	— 0.605	0.010	— 0.595	0.430	0.790	10.49
240	20.0	— 0.490	0.010	— 0.480	0.545	0.675	10.71
300	19.5	— 0.420	0.015	— 0.405	0.620	0.600	10.27

mean value... 10.9

$$k/e \cdot (\text{sal. f.}) = 11.3 \cdot 10^{-2}.$$

As k_{30}/k_{20} is 2.0, the heat of activation is 12200 cal. The enzymic hydrolysis of propylglucoside not having been investigated previously, we have examined whether the rate of hydrolysis is proportional to the concentration of emulsin. Table III shows this to be the case.

Table III.

Proportionality between rate of hydrolysis and enzymic concentration.

$c_{emulsin}$	$c_{glucoside}$	$k \cdot 10^4$	$k/e \cdot (\text{sal. f.})$
0.0665 g	0.0400 M	7.05	$24.1 \cdot 10^{-2}$
0.1330 g	0.0400 M	14.10	$24.1 \cdot 10^{-2}$
0.1995 g	0.0400 M	20.65	$23.5 \cdot 10^{-2}$

b. The affinity constant and its temperature co-efficient.

The affinity constant has been determined at 30° and at 20°. Tables IV and V and fig. 1 show that at both temperatures the affinity constant is 6.3, so that the heat of formation for the glucoside-emulsin-compound is zero.

Table IV.
Affinity constant. 30°.

$c_{emulsin} = 0.2200$ g in 50 ml. $\alpha_{emulsin} = -0.430°$ in all experiments.

Time min.	Samples kept h.	α	corr.	α corr.	x	1/v
I. 0.0200 M glucoside. 1/c = 50.						
0	3.0	−0.730	—	−0.730	—	—
20	3.0	−0.655	—	−0.655	0.075	13.35
40	4.0	−0.590	—	−0.590	0.140	7.14
60	4.5	−0.535	—	−0.535	0.195	5.12
80	5.5	−0.480	—	−0.480	0.250	4.00
II. 0.0400 M glucoside. 1/c. = 25.						
0	3.0	−1.030	—	−1.030	—	—
20	3.5	−0.925	—	−0.925	0.105	9.53
40	4.0	−0.805	—	−0.805	0.225	4.44
60	5.0	−0.695	—	−0.695	0.335	2.98
80	5.5	−0.610	—	−0.610	0.420	2.38
III. 0.0800 M glucoside. 1/c = 12.5.						
0	3.0	−1.630	—	−1.630	—	—
20	3.5	−1.455	—	−1.455	0.175	5.71
40	4.0	−1.290	—	−1.290	0.340	2.94
60	5.0	−1.125	0.005	−1.120	0.510	1.96
80	6.0	−0.970	0.005	−0.965	0.665	1.53
IV. 0.1600 M glucoside. 1/c = 6.25.						
0	3.5	−2.830	—	−2.830	—	—
20	3.5	−2.580	—	−2.580	0.250	4.00
40	4.5	−2.315	—	−2.315	0.515	1.94
60	5.0	−2.085	0.005	−2.080	0.750	1.33
80	6.0	−1.880	0.005	−1.875	0.955	1.05
V. 0.2400 M glucoside. 1/c = 4.17.						
0	3.5	−4.030	—	−4.030	—	—
20	4.0	−3.695	—	−3.695	0.335	2.98
40	4.5	−3.400	0.005	−3.395	0.635	1.58
60	5.5	−3.135	0.005	−3.130	0.900	1.11
80	6.0	−2.870	0.010	−2.860	1.170	0.85
VI. 0.3200 M glucoside. 1/c = 3.13.						
0	3.5	−5.230	—	−5.230	—	—
20	4.0	−4.840	—	−4.840	0.390	2.56
40	4.5	−4.520	0.005	−4.515	0.715	1.40
60	5.5	−4.210	0.005	−4.205	1.025	0.98
80	6.0	−3.905	0.010	−3.895	1.335	0.75

Table V.
Affinity constant. 20°.

$c_{emulsin} = 0.2000$ g in 50 ml. $\alpha_{emulsin} = -0.390°$ in all experiments.

| Time min.	Samples kept h.	α	corr.	α corr.	x	1/v
I. 0.0200 M glucoside. 1/c = 50.0.						
0	3.0	— 0.690	—	— 0.690	—	—
20	3.5	— 0.660	—	— 0.660	0.030	33.33
40	5.0	— 0.625	—	— 0.625	0.065	15.40
60	5.5	— 0.595	—	— 0.595	0.095	10.52
80	6.5	— 0.565	—	— 0.565	0.125	8.00
II. 0.400 M glucoside. 1/c = 25.0.						
0	3.5	— 0.990	—	— 0.990	—	—
20	4.0	— 0.930	—	— 0.930	0.060	16.67
40	5.0	— 0.875	—	— 0.875	0.115	8.71
60	6.0	— 0.825	—	— 0.825	0.165	6.06
80	6.5	— 0.780	—	— 0.780	0.210	4.76
III. 0.0800 M glucoside. 1/c = 12.5.						
0	3.5	— 1.590	—	— 1.590	—	—
20	4.0	— 1.500	—	— 1.500	0.090	11.11
40	5.0	— 1.415	—	— 1.415	0.175	5.72
60	6.0	— 1.345	—	— 1.345	0.245	4.08
80	6.5	— 1.270	—	— 1.270	0.320	3.13
IV. 0.1600 M glucoside. 1/c = 6.25.						
0	3.5	— 2.790	—	— 2.790	—	—
20	4.0	— 2.655	—	— 2.655	0.135	7.41
40	5.5	— 2.525	—	— 2.525	0.265	3.77
60	6.0	— 2.405	0.005	— 2.400	0.390	2.56
80	7.0	— 2.295	0.005	— 2.290	0.500	2.00
V. 0.2400 M glucoside. 1/c = 4.17.						
0	4.0	— 3.990	—	— 3.990	—	—
20	4.5	— 3.820	—	— 3.820	0.170	5.89
40	5.5	— 3.655	—	— 3.655	0.335	2.98
60	6.5	— 3.495	0.005	— 3.490	0.500	2.00
80	7.0	— 3.350	0.005	— 3.345	0.645	1.55
VI. 0.3200 M glucoside. 1/c = 3.13.						
0	4.5	— 5.190	—	— 5.190	—	—
20	5.0	— 5.015	—	— 5.015	0.175	5.72
40	5.5	— 4.830	—	— 4.830	0.360	2.78
60	6.5	— 4.650	0.005	— 4.645	0.545	1.94
80	7.0	— 4.480	0.005	— 4.475	0.715	1.40
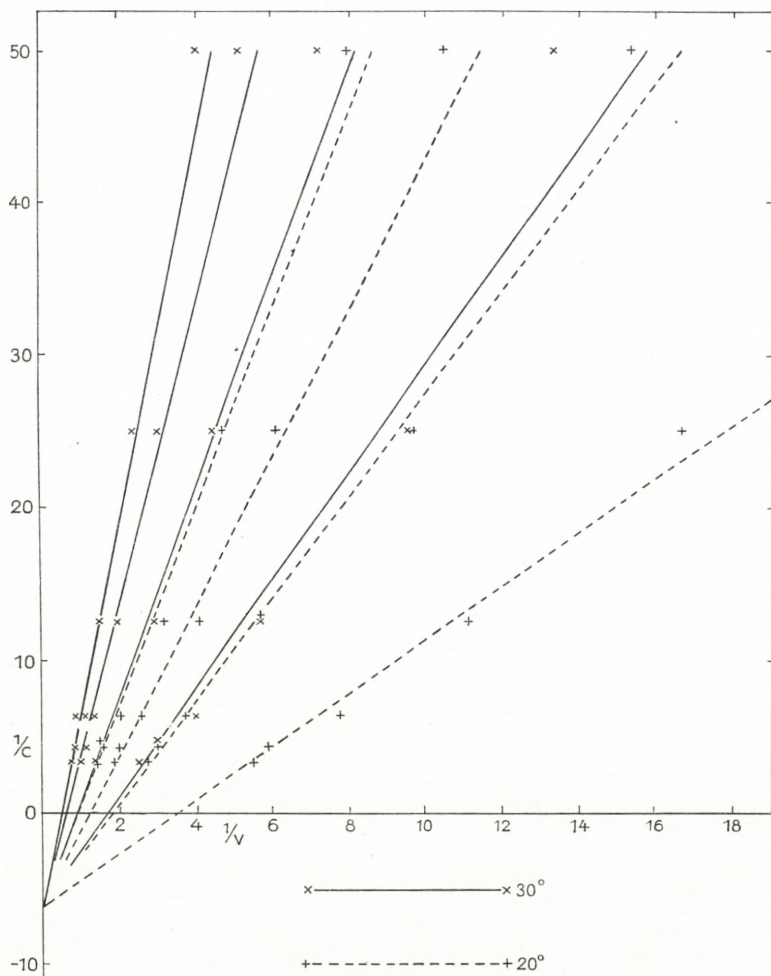
Fig. 1.

c. The influence of the products of hydrolysis.

As in the previous paper the influence of the products of hydrolysis was determined by hydrolysing solutions, which were 0.0400 M in glucoside and at the same time 0, 0.01, 0.02, 0.04, 0.08 or 0.12 M in glucose or in propyl alcohol. Tables VI and VII show the influence of glucose and propyl

alcohol respectively, tables VIII—XI are examples showing the variations within single experiments.

Table VI.
Influence of glucose.
$c_{emulsin}$ and $c_{glucoside}$ as in table VIII. 1 ml toluene added.

$c_{glucose}$	0.00	0.01	0.02	0.04	0.08	0.12 M
$k \cdot 10^4$	28.0	27.2	25.3	24.2	21.9	20.0

Table VII.
Influence of propyl alcohol.
$c_{emulsin}$ and $c_{glucoside}$ as in table X. No toluene added.

$c_{alcohol}$	0.00	0.01	0.02	0.04	0.08	0.12 M
$k \cdot 10^4$	24.9	22.6	21.3	19.8	18.5	16.7

Table VIII.
Influence of glucose. 1 ml toluene added.
$c_{emulsin}$ 0.2290 g in 50 ml. $\alpha_{emulsin} = -0.435°$. Sal. f. $= 0.044$. $c_{glucoside} = 0.0400$ M. $c_{glucose} = 0.0200$ M. $\alpha_{beg.} = -0.720°$, $\alpha_{end} = +0.500°$.

Time min.	Samples kept h.	α	corr.	α corr.	x	c — x	$k \cdot 10^4$
0	4.0	— 0.725	0.005	— 0.720	—	1.220	—
20	5.0	— 0.590	0.005	— 0.585	0.135	1.085	25.46
40	6.0	— 0.470	0.005	— 0.465	0.255	0.965	25.46
60	7.0	— 0.360	0.005	— 0.355	0.365	0.855	25.73
90	9.5	— 0.225	0.010	— 0.215	0.505	0.715	25.78
120	10.0	— 0.105	0.015	— 0.090	0.630	0.590	26.29
180	24.5	+ 0.030	0.035	+ 0.065	0.785	0.435	24.88
240	24.5	+ 0.145	0.040	+ 0.185	0.905	0.315	24.50
300	24.5	+ 0.230	0.040	+ 0.270	0.990	0.230	24.15
						mean value...	25.3

In this case both components of the glucoside are acting as inhibitors, the alcohol perhaps to a somewhat greater extent than the glucose. From tables XII and XIII it is seen that the affinity-constant for the emulsin-glucose-

Table IX.

Influence of glucose. 1 ml. toluene added.

$c_{emulsin}$ and $c_{glucoside}$ as in table VIII. $c_{glucose}$ 0.1200 M.

$$\alpha_{beg.} = +0.820°, \quad \alpha_{end} = +2.040°.$$

Time min.	Samples kept h.	α	corr.	α corr.	x	c — x	$k \cdot 10^4$
0	4.5	+ 0.800	0.020	+ 0.820	—	1.220	—
20	6.0	+ 0.895	0.025	+ 0.920	0.100	1.120	18.57
40	6.5	+ 1.000	0.025	+ 1.025	0.205	1.015	19.96
60	7.5	+ 1.085	0.030	+ 1.115	0.295	0.925	20.04
90	10.0	+ 1.205	0.045	+ 1.250	0.430	0.790	20.97
120	10.0	+ 1.310	0.045	+ 1.355	0.535	0.685	20.91
180	25.0	+ 1.395	0.115	+ 1.510	0.690	0.530	20.12
240	25.0	+ 1.515	0.120	+ 1.635	0.815	0.405	19.95
300	25.0	+ 1.600	0.120	+ 1.720	0.900	0.320	19.37
						mean value...	20.0

Table X.

Influence of propyl alcohol. No toluene added.

$c_{emulsin} = 0.2308$ g in 50 ml. $\alpha_{emulsin} = -0.450°$.

Sal. f. = 0.044. $c_{glucoside} = 0.0400$ M. $c_{alcohol} = 0.02$ M.

$$\alpha_{beg.} = -1.050°, \quad \alpha_{end} = +0.170°.$$

Time min.	Samples kept h.	α	corr.	α corr.	x	c — x	$k \cdot 10^4$
0	4.0	— 1.050	—	— 1.050	—	1.220	—
20	4.0	— 0.935	—	— 0.935	0.115	1.105	21.5
40	5.0	— 0.830	—	— 0.830	0.220	1.000	21.6
60	5.5	— 0.745	—	— 0.745	0.305	0.915	20.8
90	6.0	— 0.625	0.005	— 0.620	0.430	0.790	20.9
120	22.0	— 0.520	0.015	— 0.505	0.545	0.675	21.4
180	24.5	— 0.345	0.020	— 0.325	0.725	0.495	21.8
240	24.5	— 0.230	0.020	— 0.210	0.840	0.380	21.1
300	24.5	— 0.140	0.025	— 0.115	0.935	0.285	21.1
						mean value...	21.3

compound is $K_{m1} = 0.21$; $K_{M1} = 4.8$, the affinity constant for the propylalcohol-emulsin-compound is $K_{m2} = 0.18$; $K_{M2} = 5.6$.

Table XI.

Influence of propyl alcohol. No toluene added.

$c_{emulsin}$ and $c_{glucoside}$ as in table X. $c_{alcohol} = 0.1200$ M.

$\alpha_{beg.} = -1.050°$, $\alpha_{end} = +0.170°$.

Time min.	Samples kept h.	α	corr.	α corr.	x	c — x	$k \cdot 10^4$
0	5.0	— 1.050	—	— 1.050	—	1.220	—
20	5.0	— 0.970	—	— 0.970	0.080	1.140	14.7
40	5.5	— 0.880	—	— 0.880	0.170	1.050	15.9
60	6.0	— 0.790	—	— 0.790	0.260	0.960	17.4
90	6.5	— 0.680	0.005	— 0.675	0.375	0.845	17.8
120	22.0	— 0.600	0.010	— 0 590	0.460	0.760	17.1
180	24.5	— 0.430	0.015	— 0.415	0.635	0.585	17.7
240	24.5	— 0.335	0.020	— 0.315	0.735	0.485	16.7
300	24.5	— 0.245	0.020	— 0.225	0.825	0.395	16.3
						mean value...	16.7

The accuracy of these determinations is not very great, but it is seen that the value found here for glucose within the limits of the experiment is the same as found previously (l. c. 1936 2, p. 24).

Table XII.

Affinity constant for the compound emulsin-glucose.

$K_m = 0.16$, $S = 0.04$, $G = 0.01 - 0.12$.

G..	0.00	0.01		0.02		0.04		0.08		0.12	
t	v_0	v	v_0/v	v	v_0/v	v	v_0/v	v	v_0/v	v	v_0/v
20	0.155	0.150	1.035	0.135	1.150	0.130	1.192	0.120	1.293	0.100	1.550
40	0.285	0.275	1.038	0.255	1.119	0.250	1.142	0.225	1.268	0.205	1.392
60	0.405	0.385	1.053	0.365	1.112	0.360	1.128	0.310	1.306	0.295	1.374
90	0.555	0.545	1.019	0.505	1.100	0.485	1.144	0.455	1.221	0.430	1.290
mean value...			1.036		1.120		1.152		1.272		1.402
K_{m_1}			0.222		0.133		0.211		0.237		0.239
mean value...						$K_{m1} = 0.21$, $K_{M1} = 4.8$					

Table XIII.

Affinity constant for the compound alcohol-emulsin.

$$K_m = 0.16, \quad S = 0.04, \quad G = 0.01 - 0.12.$$

G..	0.00	0.01		0.02		0.04		0.08		0.12
t	v_0	v	v_0/v	v	v_0/v	v	v_0/v	v	v_0/v	v_0/v
20	0.125	0.120	1.041	0.115	1.086	0.105	1.190	0.100	1.250	0.080	1.568
40	0.250	0.230	1.086	0.220	1.135	0.205	1.220	0.185	1.352	0.170	1.472
60	0.350	0.320	1.094	0.305	1.150	0.285	1.230	0.280	1.250	0.260	1.350
90	0.475	0.450	1.055	0.430	1.104	0.420	1.131	0.395	1.204	0.375	1.266
mean value...		1.069		1.119		1.193		1.264		1.414
K_{m_2}		0.116		0.135		0.166		0.242		0.232

mean value... $K_{m2} = 0.18, \quad K_{M2} = 5.6$

2. Isopropyl-β-d-glucoside.

The preparation of isopropylglucoside used here had M. P. $128-29°$ and $[\alpha]_D^{20} = -40.9°$ (VEIBEL and ERIKSEN, 1936, 1).

Table XIV.

Hydrolysis at 30°.

$$c_{emulsin} = 0.1845 \text{ g in } 50 \text{ ml.} \quad \alpha_{emulsin} = -0.360°,$$
$$\text{Sal. f.} = 0.044. \quad c_{glucoside} = 0.0400 \text{ M.} \quad \alpha_{beg.} = -0.625°.$$
$$\alpha_{end} = +0.620°.$$

Time min.	Samples kept h.	α	corr.	α corr.	x	$c - x$	$k \cdot 10^4$
0	4.0	-0.985	—	-0.985	—	1.245	—
30	4.5	-0.860	—	-0.860	0.125	1.120	15.32
60	4.5	-0.770	—	-0.770	0.215	1.030	13.72
90	5.0	-0.680	—	-0.680	0.305	0.940	13.56
120	4.5	-0.600	—	-0.600	0.385	0.860	13.39
180	4.0	-0.485	—	-0.485	0.500	0.745	12.39
240	3.5	-0.395	—	-0.395	0.590	0.655	(11.62)
300	20.0	-0.320	0.015	-0.305	0.680	0.565	(11.44)
360	19.5	-0.260	0.015	-0.245	0.740	0.505	(10.88)

mean value...   13.7

$$k/e \cdot (\text{sal. f.}) = 16.9 \cdot 10^{-2}.$$

Table XV.

Hydrolysis at 20°.

c_{emulsin} and $c_{\text{glucoside}}$ as in table XIV.

Time min.	Samples kept h.	α	corr.	α corr.	x	c — x	$k \cdot 10^4$
0	4.0	— 0.985	—	— 0.985	—	1.245	—
30	4.5	— 0.925	—	— 0.925	0.060	1.185	7.15
60	5.0	— 0.875	—	— 0.875	0.110	1.135	6.70
90	5.0	— 0.820	—	— 0.820	0.165	1.080	6.86
120	5.0	— 0.780	—	— 0.780	0.205	1.040	6.51
180	4.0	— 0.715	—	— 0.715	0.270	0.975	5.90
240	3.5	— 0.645	—	— 0.645	0.340	0.905	5.77
300	20.5	— 0.600	0.010	— 0.590	0.395	0.850	5.89
360	20.0	— 0.550	0.010	— 0.540	0.445	0.800	(5.34)
						mean value...	6.4

$$k/e \cdot (\text{sal. f.}) = 7.9 \cdot 10^{-2}.$$

a. Determination of the velocity constant at 30° and its temperature coefficient.

Tables XIV and XV give the results of hydrolysis experiments at 30° and at 20°.

It is seen that $k_{30} / k_{20} = 2.15$, and consequently the heat of activation is 13500 cal.

As in the case of propyl-glucoside we have examined whether proportionality exists between concentration of emulsin and rate of hydrolysis. Table XVI shows that this is the case.

Table XVI.

Proportionality between rate of hydrolysis and concentration of emulsin.

c_{emulsin}	$c_{\text{glucoside}}$	$k \cdot 10^4$	$k/e \cdot (\text{sal. f.})$
0.0845 g	0.0400 M	5.9	$15.9 \cdot 10^{-2}$
0.1690 g	0.0400 M	11.9	$16.0 \cdot 10^{-2}$
0.2535 g	0.0400 M	17.6	$15.8 \cdot 10^{-2}$

Scheiber (1935) has examined the hydrolysis of isopropyl-glucoside, but his results are not directly comparable with

Table XVII.
Affinity constant. 30°.
$$c_{emulsin} = 0.2260 \text{ g in 50 ml. } \alpha_{emulsin} = -0.440°$$
in all experiments.

Time min.	Samples kept h.	α	corr.	α corr.	x	1/v
I. 0.200 M glucoside. 1/c = 50.0.					
0	3.5	— 0.755	—	— 0.755	—	—
30	3.5	— 0.675	—	— 0.675	0.080	12.50
60	4.0	— 0.610	—	— 0.610	0.145	6.90
90	5.0	— 0.560	—	— 0.560	0.195	5.13
120	6.0	— 0.510	—	— 0.510	0.245	4.08
II. 0.0400 M glucoside. 1/c = 25.0.					
0	3.5	— 1.060	—	— 1.060	—	—
30	4.0	— 0.920	—	— 0.920	0.140	7.14
60	4.5	— 0.805	—	— 0.805	0.255	3.92
90	5.5	— 0.705	—	— 0.705	0.355	2.82
120	6.0	— 0.620	0.005	— 0.615	0.445	2.25
III. 0.0800 M glucoside. 1/c = 12.5.					
0	3.5	— 1.690	—	— 1.690	—	—
30	4.0	— 1.480	—	— 1.480	0.210	4.76
60	4.5	— 1.250	—	— 1.250	0.440	2.27
90	5.5	— 1.050	0.005	— 1.045	0.645	1.55
120	6.0	— 0.880	0.005	— 0.875	0.815	1.23
IV. 0.1600 M glucoside. 1/c = 6.25.					
0	3.5	— 2.940	—	— 2.940	—	—
30	4.0	— 2.535	—	— 2.535	0.405	2.47
60	4.5	— 2.150	0.005	— 2.145	0.795	1.26
90	5.5	— 1.825	0.005	— 1.820	1.120	0.89
120	6.0	— 1.525	0.010	— 1.515	1.425	0.70
V. 0.2400 M glucoside. 1/c = 4.17.					
0	3.5	— 4.190	—	— 4.190	—	—
30	4.0	— 3.660	—	— 3.660	0.530	1.89
60	5.5	— 3.150	0.005	— 3.145	1.045	0.96
90	6.0	— 2.700	0.010	— 2.690	1.500	0.67
120	6.0	— 2.320	0.010	— 2.310	1.880	0.53
VI. 0.3200 M glucoside. 1/c = 3.13.					
0	4.0	— 5.440	—	— 5.440	—	—
30	4.5	— 4.780	0.005	— 4.775	0.665	1.50
60	5.5	— 4.165	0.005	— 4.160	1.280	0.78
90	6.0	— 3.620	0.010	— 3.610	1.830	0.55
120	6.5	— 3.125	0.015	— 3.110	2.330	0.43

Table XVIII.

Affinity constant. 20°.

$$c_{emulsin} = 0.2520 \text{ g in 50 ml. } \alpha_{emulsin} = -0.495°$$

in all experiments.

| Time min.	Samples kept h.	α	corr.	α corr.	x	1/c
I. 0.0200 M glucoside. 1/c = 50.0.						
0	4.0	− 0.810	—	− 0.810	—	—
30	4.5	− 0.765	—	− 0.765	0.045	22.22
60	6.0	− 0.730	—	− 0.730	0.080	12.50
90	7.0	− 0.695	—	− 0.695	0.115	8.70
120	9.5	− 0.660	—	− 0.660	0.150	6.67
II. 0.0400 M glucoside. 1/c = 25.0.						
0	4.0	− 1.120	—	− 1.120	—	—
30	4.5	− 1.050	—	− 1.050	0.070	14.30
60	6.5	− 0.975	—	− 0.975	0.145	6.90
90	7.0	− 0.920	—	− 0.920	0.200	5.00
120	9.5	− 0.870	0.005	− 0.865	0.255	3.92
III. 0.0800 M glucoside. 1/c = 12.5.						
0	4.0	− 1.745	—	− 1.745	—	—
30	5.5	− 1.605	—	− 1.605	0.140	7.14
60	6.5	− 1.460	—	− 1.460	0.285	3.51
90	7.0	− 1.360	0.005	− 1.355	0.390	2.56
120	10.0	− 1.270	0.005	− 1.265	0.480	2.08
IV. Glucoside 0.1600 M. 1/c = 6.25.						
0	4.5	− 2.995	—	− 2.995	—	—
30	6.0	− 2.765	—	− 2.765	0.230	4.35
60	6.5	− 2.560	0.005	− 2.555	0.440	2.27
90	7.0	− 2.375	0.005	− 2.370	0.625	1.60
120	10.0	− 2.185	0.010	− 2.175	0.820	1.22
V. 0.2400 M glucoside. 1/c = 4.17.						
0	4.5	− 4.245	—	− 4.245	—	—
30	6.0	− 3.955	—	− 3.955	0.290	3.45
60	7.0	− 3.630	0.005	− 3.625	0.620	1.61
90	7.5	− 3.370	0.005	− 3.365	0.880	1.14
120	10.0	− 3.130	0.010	− 3.120	1.125	0.89
VI. 0.3200 M glucoside. 1/c = 3.13.						
0	4.5	− 5.495	—	− 5.495	—	—
30	6.5	− 5.135	0.005	− 5.130	0.365	2.74
60	7.0	− 4.765	0.005	− 4.760	0.735	1.36
90	7.5	− 4.410	0.010	− 4.400	1.095	0.91
120	10.5	− 4.120	0.015	− 4.105	1.390	0.72
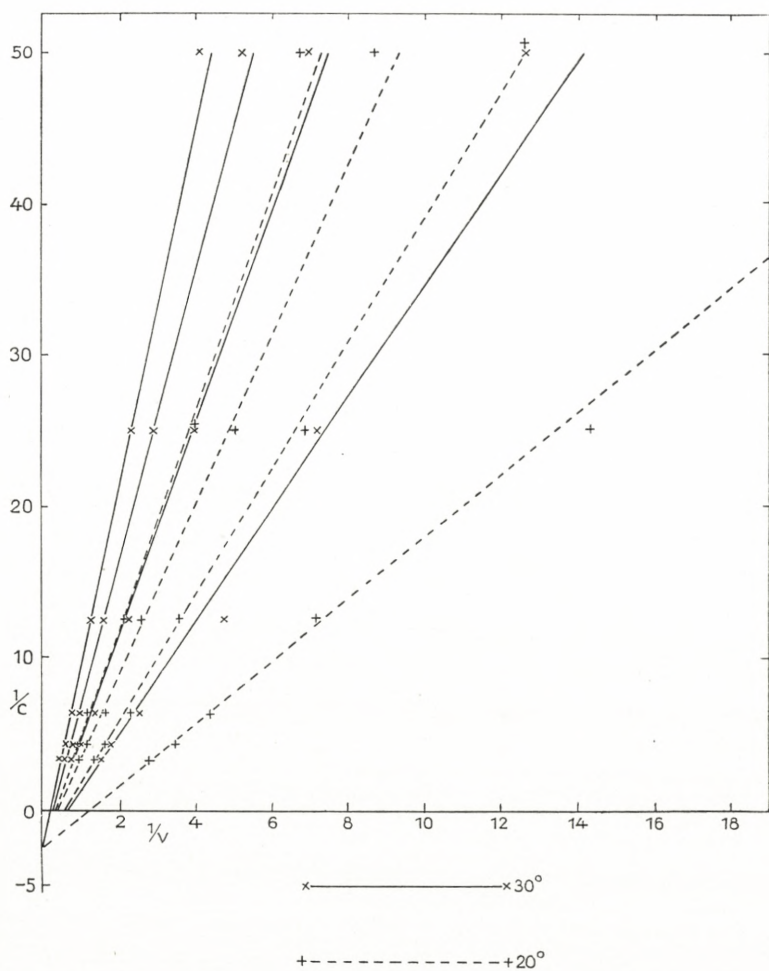
Fig. 2.

ours, his experiments having been carried out at p_H 5, whereas we are working at p_H 4.4. Nevertheless we have calculated the results reported by SCHEIBER in our way. The value of k/e·(sal. f.) for a 0.052 M solution is $11.7 \cdot 10^{-2}$, and we think that this value agrees tolerably with the value $15.9 \cdot 10^{-2}$ found by us for a 0.0400 M solution.

Table XIX.

Influence of glucose.

$c_{emulsin}$ and $c_{glucoside}$ as in table XXI. 1 ml toluene added.

$c_{glucose}$	0.00	0.01	0.02	0.04	0.08	0.12
$k \cdot 10^4$	18.3	17.5	15.7	14.3	11.2	9.5

Table XX.

Influence of isopropylalcohol.

$c_{emulsin}$ and $c_{glucoside}$ as in table XXIII. No toluene added.

$c_{alcohol}$	0.00	0.01	0.02	0.04	0.08	0.12
$k \cdot 10^4$	15.3	14.7	14.3	13.9	13.2	12.4

Table XXI.

Influence of glucose. 1 ml toluene added.

$c_{emulsin}$ 0.2182 g in 50 ml. $\alpha_{emulsin} = -0.430°$. sal. f. $= 0.044$
$c_{glucoside}$ 0.0400 M. $c_{glucose}$ 0.0200 M. $\alpha_{beg.} -0.740°$, $\alpha_{end} +0.505°$.

Time min.	Samples kept h.	α	corr.	α corr.	x	c — x	$k \cdot 10^4$
0	4.5	— 0.745	0.005	— 0.740	—	1.245	—
30	5.0	— 0.590	0.005	— 0.585	0.155	1.095	18.59
60	6.0	— 0.485	0.005	— 0.480	0.260	0.985	16.96
90	6.0	— 0.380	0.005	— 0.375	0.365	0.880	16.74
120	6.5	— 0.315	0.005	— 0.310	0.430	0.815	15.33
180	22.0	— 0.190	0.025	— 0.165	0.575	0.670	14.95
240	21.5	— 0.075	0.030	— 0.045	0.695	0.550	14.78
300	21.0	+ 0.015	0.030	+ 0.045	0.785	0.460	14.41
360	21.5	+ 0.080	0.035	+ 0.115	0.855	0.390	14.00
					mean value...		15.7

b. The affinity constant and its temperature coefficient.

The affinity constant has been determined at 30° and at 20°. Tables XVII and XVIII and fig. 2 show that at both temperatures the affinity constant is 2.5, so that the heat of formation of the glucoside-emulsin-compound is zero.

Table XXII.

Influence of glucose. 1 ml toluene added.

$c_{emulsin}$ and $c_{glucoside}$ as in table XXI. $c_{glucose}$ 0.1200 M. $\alpha_{beg} = +0.800°$. $\alpha_{end} = +2.045°$.

Time min.	Samples kept h.	α	corr.	α corr.	x	c — x	x · 10⁴
0	4.5	+ 0.780	0.020	+ 0.800	—	1.245	—
30	6.0	+ 0.855	0.025	+ 0.880	0.080	1.165	9.61
60	6.0	+ 0.940	0.025	+ 0.965	0.165	1.080	10.29
90	6.5	+ 1.020	0.030	+ 1.050	0.250	0.995	10.82
120	6.5	+ 1.075	0.030	+ 1.105	0.305	0.940	10.17
180	22.0	+ 1.095	0.095	+ 1.190	0.390	0.855	9.07
240	22.0	+ 1.180	0.095	+ 1.275	0.475	0.770	8.70
300	22.0	+ 1.255	0.100	+ 1.355	0.555	0.690	8.54
380	22.0	+ 1.350	0.100	+ 1.450	0.650	0.595	8.91

mean value... 9.5

Table XXIII.

Influence of isopropylalcohol. No toluene added.

$c_{emulsin} = 0,2250\,g$ in 50 ml. $\alpha_{emulsin} = -0.440°$. Sal. f. $= 0.044$. $c_{glucoside} = 0.0400$ M. $c_{alcohol} = 0.02$ M. $\alpha_{beg} = -1.065°$, $\alpha_{end} = +0.180°$.

Time min.	Samples kept h.	α	corr.	α corr.	x	c — x	k · 10⁴
0	4.5	— 1.065	—	— 1.065	—	1.245	—
30	5.0	— 0.940	—	— 0.940	0.125	1.120	15.32
60	6.0	— 0.825	—	— 0.825	0.240	1.005	15.57
90	6.5	— 0.740	—	— 0.740	0.325	0.920	14.60
120	6.5	— 0.660	0.005	— 0.655	0.410	0.835	14.46
180	22.5	— 0.525	0.015	— 0.510	0.555	0.690	14.24
240	22.0	— 0.420	0.015	— 0.405	0.660	0.585	13.67
300	23.0	— 0.335	0.020	— 0.315	0.750	0.495	13.35
360	22.5	— 0.265	0.020	— 0.245	0.820	0.425	12.97

mean value... 14.3

c. The influence of the products of hydrolysis.

As usual the hydrolysis of solutions being 0.04 M in glucoside and besides 0.00, 0.01, 0.02, 0.04, 0.08 or 0.12 M in glucose or in alcohol was examined. Tables XIX and XX

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Table XXIV.

Influence of isopropylalcohol. No toluene added.

$c_{emulsin}$ and $c_{glucoside}$ as in table XXIII. $c_{alcohol}$ 0.1200 M.

Time min.	Samples kept h.	α	corr.	α corr.	x	c — x	$k \cdot 10^4$
0	4.5	— 1.065	—	— 1.065	—	1.245	—
30	6.0	— 0.965	—	— 0.965	0.100	1.145	12.12
60	6.0	— 0.865	—	— 0.865	0.200	1.045	12.68
90	6.5	— 0.770	—	— 0.770	0.295	0.950	13.05
120	6.5	— 0.690	0.005	— 0.685	0.380	0.865	13.18
180	22.5	— 0.560	0.010	— 0.550	0.515	0.730	12.88
240	22.5	— 0.470	0.015	— 0.455	0.610	0.635	12.18
300	23.0	— 0.380	0.015	— 0.365	0.700	0.545	11.96
360	22.5	— 0.320	0.015	— 0.305	0.760	0.485	11.37
						mean value...	12.4

Table XXV.

Affinity constant for the compound emulsin-glucose.

$K_m = 0.40$, S = 0.04, G = 0.01 — 0.12.

G	0.00		0.01		0.02		0.04		0.08		0.12
t	v_0	v	v_0/v	v	v_0/v	v	v_0/v	v	v_0/v	v	v_0/v
30	0.170	0.160	1.062	0.155	1.096	0.135	1.259	0.100	1.700	0.080	2.130
60	0.305	0.295	1.033	0.260	1.172	0.245	1.243	0.185	1.650	0.165	1.850
90	0.425	0.415	1.026	0.365	1.165	0.340	1.250	0.265	1.605	0.250	1.702
120	0.500	0.485	1.032	0.430	1.162	0.405	1.237	0.325	1.540	0.305	1.640
mean value		1.038		1.149		1.247		1.624		1.831	
K_{m1}		0.240		0.122		0.147		0.117		0.131	
mean value		$K_{m1} = 0.15$; $K_{M1} = 6.6$									

show respectively the influence of glucose and isopropyl-alcohol, Tables XXI—XXIV are examples showing the variations within single experiments.

Here also both components act as inhibitors. By comparison with propylglucoside it is seen that isopropylalcohol does not inhibit as much as does propylalcohol. Tables XXV and XXVI give material for the calculation of the

Table XXVI.

Affinity constant for the compound emulsin-isopropylalcohol.

$$K_m = 0.40, \ S = 0.04, \ G = 0.01 - 0.12.$$

G	0.00	0.01		0.02		0.04		0.08		0.12	
t	v_0	v	v_0/v	v	v_0/v	v	v_0/v	v	v_0/v	v	v_0/v
30	0.135	0.130	1.035	0.125	1.070	0.120	1.125	0.115	1.175	0.100	1.350
60	0.250	0.245	1.022	0.240	1.045	0.235	1.065	0.225	1.115	0.200	1.250
90	0.345	0.335	1.030	0.325	1.062	0.320	1.080	0.305	1.135	0.295	1.170
120	0.435	0.420	1.038	0.410	1.062	0.405	1.078	0.395	1.105	0.380	1.150
mean value			1.03		1.06		1.09		1.13		1.23
K_{m1}			0.30		0.30		0.40		0.58		0.47

mean value $K_{m1} = 0.41; \ K_{M1} = 2.5$

affinity constants of glucose and isopropylalcohol. The values found for glucose are $K_{m1} = 0.15; \ K_{M1} = 6.6$ (i. e. within the limits of the experiment the same value as found previously), for isopropylalcohol $K_{m2} = 0.41; \ K_{M2} = 2.5$.

Discussion.

We have now established the following constants for methyl-, ethyl-, propyl- and isopropyl-β-d-glucoside:

Table XXVII.

M = 0.0400	Methyl	Ethyl	Propyl	Isopropyl
$10^2 \cdot k_{30}/e \cdot$ (sal. f.)	2.7	5.3	22.6	16.9
K_m	0.62	0.25	0.16	0.40
K_{m1}	0.21	0.17	0.21	0.15
K_{m2}	—	—	0.18	0.41

The mean value for K_{m1} (glucose) is 0.18. The K_{m2}-values for methyl- and ethylalcohol are so small that we have not been able to determine them with the experimental technique used here.

JOSEPHSON (1925, p. 145) has found that for some of the glucosides examined by him the equation $K_m = K_{m1} \cdot K_{m2}$ holds good. We have not been able to determine the K_{m2}-

values for methyl- and ethylalcohol, and consequently we cannot determine the product $K_{m1} \cdot K_{m2}$ for these two gluco- sides. For propyl- and isopropyl-glucoside JOSEPHSON's equa- tion surely does not hold good, as will be seen from the following figures:

$$\text{Propyl: } K_m = 0.16; \; K_{m1} = 0.18; \; K_{m2} = 0.18;$$
$$K_{m1} \cdot K_{m2} = 0.032$$
$$\text{Isopropyl: } K_m = 0.40; \; K_{m1} = 0.18; \; K_{m2} = 0.41;$$
$$K_{m1} \cdot K_{m2} = 0.074.$$

It must, however, be kept in mind that JOSEPHSON divides the inhibiting action of the alcohols into two parts, one, which is independent of the affinity between the gluco- sidase and the alcohol, and another, which is due to this affinity, and it is only this part of the inhibiting effect which is to be taken into account in the calculation of the K_{m2}-values used in the JOSEPHSON-expression. Our ex- periments do not afford material for the determination of the two different effects of the alcohols, but the non-com- petitive part of the inhibiting action of the alcohols must be very great to obtain agreement between the observed and the calculated K_m-values, the latter being only some 20 % of the observed values.

Both the fact that glucose and at least two of the four alcohols examined have an inhibiting effect on the hydro- lysis of the β-glucosides with emulsin, and that it has been possible to determine the dissociation constants for the emulsin-inhibitor-compounds as well as for the emul- sin-glucoside-compounds, made us try to refer the hydro- lysis-constants not to the amount of emulsin present in the solution, as we have done till now, but to the amount of emulsin really bound to the glucoside. As the molarity

of the emulsin-solution is not known, we have calculated how many per cent. of the emulsin have combined with the glucoside, and as we have previously referred the hydrolysis-constants to the unity of emulsin present in the solution, it is not difficult to recalculate the constants, referring them to unity of emulsin combined.

From the mass-action expression

$$K_m = \frac{(E - ES)(S - ES)}{ES}$$

or, as S is always very great compared with ES,

$$K_m = \frac{(E - ES) S}{ES},$$

it is seen that $\dfrac{ES}{E} = \dfrac{S}{(K_m + S)}$.

For all four glucosides examined we have used solutions 0.0400 M in glucoside. In Table XXVIII is indicated the amount of emulsin combined with the four glucosides and the hydrolysis constants referred to unity of emulsin combined.

Table XXVIII.

Rate of hydrolysis referred to unity of emulsin combined.

	$10^2 \cdot k/(e \cdot sal.\ f.)$	K_m	% Emulsin combined	$10^2 \cdot k/(e_{comb} \cdot sal.\ f.)$
Methyl	2.7	0.62	6.1	44
Ethyl	5.3	0.25	13.8	39
Propyl	22.6	0.16	20.0	113
Isopropyl	16.9	0.40	9.1	186

It is seen that calculated in this way the order of the velocity of hydrolysis differs from the one found, when the velocity constants are referred to unity of emulsin dissolved.

It is well known that when the velocity constants of hydrolysis are determined for different substrate concentrations, no proportionality exists between k and c. If the

Table XXIX.

Constancy of the expression $k/(K_m + c)$.

c	K_m	% emulsin combined	k/(e·sal.f.)	k/e_{comb}	$c \cdot k/e_{comb}$ = $k(K_m + c)$
			Methylglucoside		
0.0204	0.62	3.1	$2.8 \cdot 10^{-2}$	$90.5 \cdot 10^{-2}$	$1.80 \cdot 10^{-2}$
0.0409	0.62	6.1	2.8	45.9	1.85
0.0826	0.62	11.7	2.6	22.2	1.82
0.1672	0.62	21.3	2.4	11.2	1.87
0.3334	0.62	35.0	2.0	5.7	1.90
0.6689	0.62	51.8	1.4	2.7	1.81
					1.84
			Ethylglucoside		
0.0200	0.25	7.4	$5.4 \cdot 10^{-2}$	$73.0 \cdot 10^{-2}$	$1.46 \cdot 10^{-2}$
0.0400	0.25	13.8	5.8	42.0	1.68
0.0800	0.25	24.3	5.1	21.0	1.68
0.1600	0.25	39.0	4.1	10.5	1.68
0.3200	0.25	56.2	2.9	5.2	1.66
0.6400	0.25	71.9	1.7	2.4	1.54
					1.62
			Propylglucoside		
0.0200	0.16	11.2	$29.2 \cdot 10^{-2}$	$260 \cdot 10^{-2}$	$5.20 \cdot 10^{-2}$
0.0400	0.16	20.0	22.7	114	4.56
0.0800	0.16	33.3	17.2	51.6	4.13
0.1600	0.16	50.0	12.4	24.8	3.97
0.2400	0.16	60.0	10.0	16.7	4.00
0.3200	0.16	66.7	8.5	12.8	4.10
					4.33
			Isopropylglucoside		
0.0200	0.40	4.8	$18.9 \cdot 10^{-2}$	$394 \cdot 10^{-2}$	$7.88 \cdot 10^{-2}$
0.0400	0.40	9.1	16.6	183	7.32
0.0800	0.40	16.7	14.5	86.8	6.94
0.1600	0.40	28.6	12.4	42.4	6.79
0.2400	0.40	37.5	11.1	29.6	7.10
0.3200	0.40	44.5	10.0	22.5	7.20
					7.21

velocity constants are referred to the emulsin combined, the constants reduced in this way are inversely proportional to the concentration of the substrate, as the measured reac-

tion seems to be the hydrolysis of the compound emulsin-substrate into emulsin, glucose and alcohol. This means that the product $k(K_m + c)$ has a constant value (k being the directly observed velocity constant).

The determination of the affinity constant of the gluco-sides affords material for the determination of the hydro-lysis constants at 6 different glucoside concentrations. In Table XXIX we have calculated the velocity constants referred to unity of the applied emulsin and we have also indicated the amount of the emulsin, which has combined with the substrate, as well as the velocity constants re-ferred to the emulsin combined and the product $k \cdot (K_m + c)$. It is seen that the concordance is as good as may be expected.

We have then examined whether the inhibiting effect of the products of hydrolysis may be explained by the affinity of emulsin to the products of hydrolysis, that is, whether the inhibition is competitive or not. The velocity constants being proportional to the concentration of emulsin, this may be examined, competitive inhibition meaning that the velocity constants of hydrolysis decrease exactly as much as the percentage of emulsin combined with glucoside de-creases by the addition of glucose or alcohol.

When the affinity constants of the compounds emulsin-glucoside and emulsin-glucose are known, it is possible to calculate, how much the amount of emulsin combined with each component decreases, both components being found together. If it is calculated for a solution, containing glu-coside and glucose, that the fraction a of the emulsin will combine with glucoside, glucose not being present, and the fraction b will combine with glucose, glucoside not being present, then the fractions x and y, which will combine

with each component, when both are present, may be calculated from the equations:

$$x = (1-y)\,a; \qquad y = (1-x)\,b$$

or

$$x = a \cdot \frac{1-b}{1-ab}.$$

If the inbibition is competitive, $\dfrac{k}{x}$ has to be constant for all experiments with the same glucoside concentration.

In Table XXX we have made this calculation for the action of glucose on the hydrolysis of all four glucosides

Table XXX.

Elimination of the effect of inhibitors.

$c_{inhibitor}$	$k \cdot 10^4$	$k \cdot 10^4/a$	b %	x %	$k \cdot 10/x$
1. Methylglucoside. Inhibitor glucose. $c_{glucoside} = 0.0400$ M.					
a = 6.1%.					
0.00	3.5	57.4	0.0	6.1	57.4
0.01	3.2	52.5	5.3	5.8	55.2
0.02	3.0	49.2	10.0	5.5	54.3
0.04	2.6	42.6	18.2	5.0	51.5
0.08	2.4	39.3	30.8	4.3	55.8
0.12	2.2	36.1	40.0	3.8	58.6
2. Ethylglucoside. Inhibitor glucose. $c_{glucoside} = 0.0400$ M.					
a = 13.8 %					
0.00	8.5	61.6	0.0	13.8	61.6
0.01	8.0	58.0	5.3	13.1	60.9
0.02	7.7	55.8	10.0	12.6	61.2
0.04	7.0	50.7	18.2	11.6	60.5
0.08	5.9	42.8	30.8	10.0	59.2
0.12	5.7	41.3	40.0	8.8	65.0
3. Propylglucoside. Inhibitor glucose. $c_{glucoside} = 0.0400$ M.					
a = 20.0 %.					
0.00	28.0	140.0	0.0	20.0	140.0
0.01	27.7	138.5	5.3	19.1	145.0
0.02	25.3	126.5	10.0	18.4	137.7
0.04	24.2	121.0	18.2	17.0	142.6
0.08	21.9	109.5	30.8	14.8	148.5
0.12	20.0	100.0	40.0	13.0	153.3

Table XXX

(continued).

Elimation of the effect of inhibitors.

$c_{inhibitor}$	$k \cdot 10^4$	$k \cdot 10^4/a$	$b \ \%$	$x \ \%$	$k \cdot 10^4/x$

4. Propylglucoside. Inhibitor propylalc. $c_{glucoside} = 0.0400$ M.
a = 20.0 %.

$c_{inhibitor}$	$k \cdot 10^4$	$k \cdot 10^4/a$	$b \ \%$	$x \ \%$	$k \cdot 10^4/x$
0.00	24.9	124.5	0.0	20.0	124.5
0.01	22.6	113.6	5.3	19.1	118.3
0.02	21.3	106.5	10.0	18.4	116.0
0.04	19.8	99.0	18.2	17.0	116.6
0.08	18.5	92.5	30.8	14.8	125.5
0.12	16.7	83.5	40.0	13.0	128.0

5. Isopropylglucoside. Inhibitor glucose. $c_{glucoside} = 0.0400$ M.
a = 9.1 %.

$c_{inhibitor}$	$k \cdot 10^4$	$k \cdot 10^4/a$	$b \ \%$	$x \ \%$	$k \cdot 10^4/x$
0.00	18.3	201.1	0.0	9.1	201.1
0.01	17.5	192.3	5.2	8.6	202.5
0.02	15.7	172.5	10.0	8.3	189.9
0.04	14.3	157.1	18.2	7.6	189.0
0.08	11.2	123.0	30.8	6.5	173.0
0.12	9.5	104.4	40.0	5.7	167.6

6. Isopropylglucoside. Inhibitor isopropylalc. $c_{glucoside} = 0.0400$ M.
a = 9.1 %.

$c_{inhibitor}$	$k \cdot 10^4$	$k \cdot 10^4/a$	$b \ \%$	$x \ \%$	$k \cdot 10^4/x$
0.00	15.3	168.1	0.0	9.1	168.1
0.01	14.7	161.6	2.4	8.9	165.1
0.02	14.3	157.1	4.7	8.7	164.2
0.04	13.9	152.8	8.9	8.4	166.3
0.08	13.2	145.1	16.2	7.7	170.6
0.12	12.4	136.2	22.6	7.2	172.4

and for the action of propyl- and isopropyl-alcohol on the hydrolysis of propyl- and isopropylglucoside respectively.

The table, although the constancy of $\frac{k}{x}$ is not excellent, shows that the figures give no evidence of a non-competitive inhibition as assumed by JOSEPHSON (1925). It is, however, to be observed that JOSEPHSON used much higher alcohol concentrations than those used in these experiments.

A consequence of the observations discussed above is that the method of calculating the hydrolysis constant used

in this and the previous papers is not the correct one. We have till now calculated the hydrolysis constants with the same c_0-value for all samples withdrawn within a single experiment. As the amount of emulsin bound to the glucoside varies within the experiment, both on account of the decrease of the glucoside concentration and on account of the increase of the concentration of glucose and alcohol (both substances being able to act as inhibitors), it seems to be more correct to calculate the velocity constants "from point to point" and to find for each point the amount of emulsin really bound to the glucoside, taking the actually existing concentrations of glucose and alcohol into consideration.

In order to get constants which are comparable, it is therefore necessarry to calculate the value of $\dfrac{k \cdot c}{x \cdot e \cdot (sal.f.)}$, where

k   is the velocity constant for the hydrolysis from c_n to c_{n+1}.

c   is the glucoside concentration c_n.

x   is the fraction of the emulsin present, which is bound to the glucoside at the concentration $\dfrac{(c_n + c_{n+1})}{2}$, considering the presence of inhibiting substances.

e   the amount of emulsin present in 50 ml of the solution.

sal. f. the enzymic power of the emulsin preparation, determined by its action on salicin.

The calculation of the value of x is somewhat more complicated when two inhibiting substances are present than when only one such substance is present.

When a, b and c are the fractions of the emulsin, which are bound to the glucoside and the two inhibiting substances,

these substances being the only ones in the solutions, and
$\underline{x}$, $\underline{y}$ and $\underline{z}$ being the fractions of the emulsin bound to
each substance, when they are all together in the solution,
then

$$x = a\,(1 - y - z)$$
$$y = b\,(1 - x - z)$$
$$z = c\,(1 - x - y)$$

or

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

and the corresponding expressions for y and z.

In Tables XXXI—XXXIV we have recalculated all
experiments mentioned in this and the previous paper. It
is seen that for each temperature the value of $\dfrac{k \cdot c}{x \cdot e \cdot (sal.\ f.)}$
is really constant within the limit of the experiment. As
always it is not allowable to compare experiments where
toluene has been added with experiments without the ad-
dition of toluene.

In a recent paper MOELWYN-HUGHES (1936; comp. CHRI-
STIANSEN (1924)) has pointed out that in a reaction

$$A + B \underset{k_1}{\overset{k_2}{\rightleftarrows}} AB; \qquad AB \overset{k_3}{\to} C + D$$
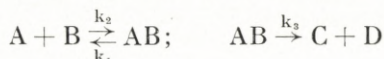
the observed velocity-constant is

$$k_{obs} = \frac{k_3 \cdot k_2}{k_1 + k_3}.$$

If $k_1 \gg k_3$ (the BRØNSTED condition)

$$k_{obs} = k_3 \cdot \frac{k_2}{k_1} = k_3 \cdot \frac{[AB]}{[A]\,[B]}$$

which is identical with the expression

$$\frac{k \cdot c}{x \cdot e\,(sal.\ f.)} = const.,$$

Table XXXI.

Methylglucoside.

$c_{glucoside}$	$c_{glucose}$	$c_{alcohol}$	k_{o-t}	$k_{t_1-t_2}$	$k \cdot c/x \cdot e\,(sal.f.)$
30° without toluene.					
0.0400	0.00	0.00	$2.56 \cdot 10^{-4}$	$2.46 \cdot 10^{-4}$	$1.86 \cdot 10^{-2}$
0.0204	0.00	0.00	2.39	2.58	2.06
0.0409	0.00	0.00	2.40	2.28	1.86
0.0826	0.00	0.00	2.20	2.15	1.90
0.1672	0.00	0.00	1.97	1.93	1.93
0.3334	0.00	0.00	1.65	1.59	1.95
0.6689	0.00	0.00	1.17	1.15	1.92
0.0400	0.00	0.00	2.67	2.54	1.92
0.0400	0.00	0.01	2.64	2.49	1.87
0.0400	0.00	0.02	2.59	2.45	1.84
0.0400	0.00	0.04	2.61	2.45	1.84
0.0400	0.00	0.08	2.58	2.42	1.82
0.0400	0.00	0.12	2.54	2.41	1.81
			mean value...		1.89
30° with addition of toluene.					
0.0400	0.00	0.00	$3.46 \cdot 10^{-4}$	$3.48 \cdot 10^{-4}$	$3.15 \cdot 10^{-2}$
0.0400	0.01	0.00	3.22	3.20	2.96
0.0400	0.02	0.00	2.98	2.81	2.75
0.0400	0.04	0.00	2.81	2.72	2.85
0.0400	0.08	0.00	2.36	2.33	2.82
0.0400	0.12	0.00	2.25	2.19	3.02
			mean value...		2.93
20° without toluene.					
0.0400	0.00	0.00	$1.39 \cdot 10^{-4}$	$1.19 \cdot 10^{-4}$	$0.87 \cdot 10^{-2}$
0.0189	0.00	0.00	1.66	1.38	1.01
0.0333	0.00	0.00	1.43	1.36	1.04
0.0769	0.00	0.00	1.26	1.10	0.89
0.1540	0.00	0.00	1.13	1.02	0.94
0.3030	0.00	0.00	0.99	0.79	0.88
0.5958	0.00	0.00	0.86	0.64	0.95
			mean value...		0.94

$k_{30}/k_{20} = 1.89/0.94 = 2.0$

Effect of toluene: $2.93/1.89 = 1.55$.

k being k_{obs} and const. $= k_3$, c $= [A]$, $x \cdot e\,(sal.\,f.) = \dfrac{[AB]}{[B]}$.

From the constancy of $\dfrac{k \cdot c}{x \cdot e\,(sal.\,f.)}$ it may be concluded that

Table XXXII.

Ethylglucoside.

$c_{glucoside}$	$c_{glucose}$	$c_{alcohol}$	k_{o-t}	$k_{t_1-t_2}$	$k \cdot c/x \cdot e$ (sal. f.)
30° without toluene.					
0.0400	0.00	0.00	$5.95 \cdot 10^{-4}$	$5.49 \cdot 10^{-4}$	$1.48 \cdot 10^{-2}$
0.0200	0.00	0.00	5.02	5.10	1.44
0.0400	0.00	0.00	5.42	5.35	1.72
0.0800	0.00	0.00	4.83	4.73	1.65
0.1600	0.00	0.00	3.77	3.80	1.64
0.3200	0.00	0.00	2.69	2.67	1.58
0.6400	0.00	0.00	1.59	1.68	1.53
0.0400	0.00	0.00	4.65	4.41	1.59
0.0400	0.00	0.01	4.70	4.47	1.61
0.0400	0.00	0.02	4.68	4.43	1.59
0.0400	0.00	0.04	4.76	4.44	1.60
0.0400	0.00	0.08	4.57	4.34	1.56
0.0400	0.00	0.12	4.56	4.36	1.56
			mean value...		1.58
30° with addition of toluene.					
0.0400	0.00	0.00	$8.16 \cdot 10^{-4}$	$7.62 \cdot 10^{-4}$	$2.27 \cdot 10^{-2}$
0.0400	0.01	0.00	7.86	7.34	2.29
0.0400	0.02	0.00	7.32	7.03	2.29
0.0400	0.04	0.00	6.87	6.36	2.25
0.0400	0.08	0.00	5.82	5.49	2.22
0.0400	0.12	0.00	5.67	5.19	2.38
			mean value...		2.28
20° without toluene.					
0.0400	0.00	0.00	$2.56 \cdot 10^{-4}$	$2.30 \cdot 10^{-4}$	$0.59 \cdot 10^{-2}$
0.0200	0.00	0.00	2.52	2.46	0.75
0.0400	0.00	0.00	2.62	2.47	0.81
0.0800	0.00	0.00	2.27	2.17	0.81
0.1600	0.00	0.00	1.77	1.70	0.80
0.3200	0.00	0.00	1.28	1.25	0.80
0.6400	0.00	0.00	0.76	0.80	0.81
			mean value...		0.77

$k_{30}/k_{20} = 1.58/0.77 = 2.05$

Effect of toluene: $2.28/1.58 = 1.44$.

the formation of the emulsin-glucoside-compound has a velocity constant which is much greater than the hydrolysis constant for its decomposition into emulsin, glucose and alcohol.

Table XXXIII.

Propylglucoside.

$c_{glucoside}$	$c_{glucose}$	$c_{alcohol}$	k_{o-t}	$k_{t_1-t_2}$	$k \cdot c/x \cdot e$ (sal. f.)
30° without toluene.					
0.0400	0.00	0.00	$21.7 \cdot 10^{-4}$	$22.3 \cdot 10^{-4}$	$5.27 \cdot 10^{-2}$
0.0200	0.00	0.00	28.4	28.6	5.78
0.0400	0.00	0.00	21.9	22.9	5.13
0.0800	0.00	0.00	16.7	17.3	4.62
0.1600	0.00	0.00	11.9	11.8	4.18
0.2400	0.00	0.00	9.8	9.5	4.14
0.3200	0.00	0.00	8.3	8.0	4.18
0.0400	0.00	0.00	24.9	24.9	5.69
0.0400	0.00	0.01	22.6	22.6	5.40
0.0400	0.00	0.02	21.3	21.1	5.20
0.0400	0.00	0.04	19.8	20.1	5.33
0.0400	0.00	0.08	18.6	18.4	5.50
0.0400	0.00	0.12	16.7	16.7	5.56
			mean value...		5.08
30° with addition of toluene.					
0.0400	0.00	0.00	$28.7 \cdot 10^{-4}$	$27.3 \cdot 10^{-4}$	$6.42 \cdot 10^{-2}$
0.0400	0.01	0.00	27.2	26.8	6.53
0.0400	0.02	0.00	25.3	24.9	6.26
0.0400	0.04	0.00	24.2	23.3	6.28
0.0400	0.08	0.00	21.9	21.4	6.53
0.0400	0.12	0.00	20.0	19.9	6.77
			mean value...		6.47
20° without toluene.					
0.0400	0.00	0.00	$10.9 \cdot 10^{-4}$	$10.7 \cdot 10^{-4}$	$2.27 \cdot 10^{-2}$
0.0200	0.00	0.00	12.0	12.5	2.65
0.0400	0.00	0.00	10.7	10.3	2.42
0.0800	0.00	0.00	7.9	7.6	2.16
0.1600	0.00	0.00	6.0	5.9	2.21
0.2400	0.00	0.00	5.1	5.0	2.35
0.3200	0.00	0.00	4.1	4.1	2.32
			mean value...		2.34

$k_{30}/k_{20} = 5.08/2.34 = 2.2$

Effect of toluene: $6.47/5.08 = 1.27$.

It is known that emulsin has not only a hydrolysing effect, but that it is also able to katalyse the synthesis of glucosides. Veibel (1936) has determined the equilibrium

Tabel XXXIV.

Isopropylglucoside.

30°. Without toluene.

$c_{glucoside}$	$c_{glucose}$	$c_{alkohol}$	k_{0-t}	$k_{t_1-t_2}$	$k \cdot c/x \cdot e$ (sal. f.)
0.0400	0.00	0.00	12.8	11.5	6.96
0.0200	0.00	0.00	18.8	17.9	8.26
0.0400	0.00	0.00	16.5	16.0	7.76
0.0800	0.00	0.00	14.0	14.4	7.72
0.1600	0.00	0.00	12.4	12.2	7.84
0.2400	0.00	0,00	11.0	10.6	7.82
0.3200	0.00	0.00	9.9	9.7	8.02
0.0400	0.00	0.00	15.3	14.4	7.32
0.0400	0.00	0.01	14.7	13.8	7.12
0.0400	0.00	0.02	14.3	13.4	7.01
0.0400	0.00	0.04	13.9	13.0	6.99
0.0400	0.00	0 08	13.2	12.4	7.27
0.0400	0.00	0.12	12.5	12.0	7.50
			mean value...		7.51

30°. With addition of toluene.

0.0400	0.00	0.00	18.4	16.5	8.79
0.0400	0.01	0.00	17.5	16.1	8.94
0.0400	0.02	0.00	15.7	14.3	8.23
0.0400	0.04	0.00	14.3	13.1	8.08
0.0400	0.08	0.00	11.2	10.6	7.44
0.0400	0.12	0.00	9.5	9.2	7.32
			mean value...		8.13

20°. Without toluene.

0.0400	0.00	0.00	6.5	5.6	3.24
0.0200	0.00	0.00	10.1	9.9	3.94
0.0400	0.00	0.00	8.52	8.25	3.43
0.0800	0.00	0.00	8.29	7.95	3.57
0.1600	0.00	0.00	6.63	6.51	3.54
0.2400	0.00	0.00	5.99	5.92	3.78
0.3200	0.00	0.00	5.50	5.44	3.85
			mean value...		3.62

$k_{30}/k_{20} = 7.51/3.62 = 2.1$

Effect of toluene: $8.13/7.51 = 1.08$

constant for mixtures of glucoside, glucose and alcohol for a series of alcohols and has found that methyl glu-

Table XXXV.

Glucoside	K_m	K_{m_1}	K_{m_2}	$k \cdot c/x \cdot e$ (sal. f.)	k_{30}/k_{20}	Effect of toluene
Methyl ...	0.62	0.18	..	$1.89 \cdot 10^{-2}$	2.0	1.55
Ethyl	0.25	0.18	..	$1.58 \cdot 10^{-2}$	2.0_5	1.44
Propyl....	0.16	0.18	0.18	$5.08 \cdot 10^{-2}$	2.1_5	1.27
Isopropyl.	0.40	0.18	0.14	$7.51 \cdot 10^{-2}$	2.1	1.08

coside is the one which has the highest equilibrium concentration, the equilibrium constant being 0.311 for a glucose-methylalcohol-water-acetone mixture with the concentrations $c_{glucose}$ 0.150 M, $c_{alcohol}$ 3.000 M, c_{water} 15.00 M at the beginning. Presuming the equilibrium constant to be the same for 0.04 M glucoside solutions in water (which is probably not quite correct), the equilibrium concentration of methyl glucoside is calculated to be 0.0002 M, i. e. only $^1/_2\%$ will not be hydrolysed. In the calculation of the velocity constants it is therefore unnecessary to consider the reversibility of the process.

Tables XXXVI—XLIII are examples showing the variations within single experiments, the constants being calculated from point to point. For each glucoside is given the experiment showing the best constancy of the constants as well as the experiment showing the greatest deviations. It is quite clear, that the constancy is not as good here as when calculating the constants from the starting point to the time t, the variations in rotation being in many cases only 0.050° and the polarimeter does not allow a greater accuracy of the readings than 0.005°. Table XLV is a recalculation of table XXXIX, the only difference being that some of the readings have been substituted by readings 0.005° (in two cases 0.010°) greater or smaller than the original ones. This table shows that the deviations from the constant value do not surpass the limits of the experiments.

It is therefore to be assumed that the mean values of the constants are correct.

In the tables the meaning of the columns is:

1 = time from the beginning

2 = α_t

3 = velocity constant calculated from t = 0 to t = t

4 = velocity constant calculated from t = n to t = n + 1

5 = $c_{glucoside}$ at time t

6 = % emulsin bound to glucoside at time t if no glucose and alcohol present

7 = % emulsin bound to glucose at time t if no glucoside and alcohol present

8 = % emulsin bound to alcohol at time t if no glucoside and glucose present

9 = % emulsin actually bound to glucoside at time t

10 = value of x

$11 = \dfrac{k}{x}$

$12 = \dfrac{k \cdot c}{x}$

Table XXXVI.

Methylglucoside 0.0400 M. Hydrolysis 30°. e = 0.2233 g in 50 ml. sal. f. = 0.043.

1	2	3	4	5	6	7	9	10	11	12
0	1.075	—	3.16	0.0400	6.06	0.00	6.06	5.78	54.7	2.19
120	0.985	3.16	2.47	0.0367	5.58	1.80	5.49	5.29	46.7	1.71
240	0.920	2.82	2.02	0.0342	5.24	3.12	5.08	4.93	41.0	1.40
360	0.870	2.55	2.33	0.0324	4.96	4.05	4.77	4.53	51.4	1.66
540	0.790	2.48	2.58	0.0294	4.53	5.56	4.29	3.98	64.9	1.91
780	0.685	2.51	2.27	0.0255	3.95	7.45	3.66	3.10	73.3	1.87
1440	0.485	2.40	2.18	0.0181	2.83	10.84	2.53	2.22	98.0	1.77
1980	0.370	2.34	1.99	0.0138	2.17	12.71	1.90	1.57	126.7	1.75
2880	0.245	2.23	. .	0.0091	1.45	14.65	1.24	. .	. .	. .
mean value		2.56	2.38							1.78

$k \cdot c / x \cdot e(\text{sal. f.}) = 1.86 \cdot 10^{-2}$

Tabel XXXVII.

Methylglucoside 0.0400 M. Hydrolysis 30°. e = 0.2259 g in
50 ml. sal. f. = 0.043.

1	2	3	4	5	6	7	9	10	11	12
0	1.075	—	2.80	0.0400	6.06	0.00	6.06	5.81	51.6	2.07
120	0.995	2.80	2.84	0.0370	5.64	1.64	5.55	5.32	53.3	1.98
240	0.920	2.82	2.65	0.0342	5.23	3.12	5.08	4.88	54.3	1.86
360	0.855	2.76	2.41	0.0318	4.88	4.36	4.68	4.52	53.3	1.70
480	0.800	2.67	2.46	0.0298	4.58	5.36	4.35	3.98	61.8	1.84
780	0.675	2.59	2.10	0.0251	3.89	7.65	3.61	3.09	68.2	1.71
1440	0.490	2.37	..	0.0182	2.86	10.80	2.56	..	..	..
mean value		2.67	2.54							1.86

$$k \cdot c/x \cdot e \cdot (\text{sal. f.}) = 1.92 \cdot 10^{-2}$$

Table XXXVIII.

Ethylglucoside 0.0400 M. Hydrolysis 30°. e = 0.2818 g in
50 ml. sal. f. = 0.044.

1	2	3	4	5	6	7	8	9	10	11	12
0	1.175	—	7.12	0.0400	13.79	0.00	—	13.79	13.12	54.3	2.18
60	1.065	7.12	6.39	0.0363	12.66	2.02	—	12.44	11.50	53.7	1.95
120	0.975	6.75	5.61	0.0332	11.72	3.64	—	11.35	10.50	53.5	1.78
240	0.835	6.18	6.95	0.0284	10.21	6.08	—	9.65	8.29	83.8	2.38
360	0.690	6.42	4.29	0.0235	8.59	8.40	—	7.92	7.37	58.2	1.37
510	0.595	5.80	5.39	0.0203	7.50	9.86	—	6.81	5.65	95.4	1.93
840	0.395	5.64	4.93	0.0134	5.11	12.88	—	4.48	3.37	146.2	1.97
1440	0.200	5.34	3.23	0.0068	2.65	15.58	—	2.25	2.02	160.0	1.09
1740	0.160	4.98	..	0.0054	2.13	16.13	—	1.79	..	..	..
mean value		6.03	5.49								1.83

$$k \cdot c/x \cdot e \, (\text{sal. f.}) = 1.48 \cdot 10^{-2}$$

Tables XXXI—XXXIV show that there is no evidence
of a non-competitive inhibition and that all experiments
agree with the assumption that the process determining the
velocity of the reaction is the hydrolysis of the addition-
compound of glucoside and emulsin into glucose, alcohol and
emulsin, the concentration of this addition compound being
determined by the values of the dissociation constants of

Table XXXIX.

Ethylglucoside. Inhibition by ethyl alcohol.

Glucoside 0.0400 M. Alcohol 0.08 M. e = 0.2008 g in 50 ml. sal. f. = 0.043.

1	2	3	4	5	6	7	8	9	10	11	12
0	1.175	—	5.11	0.0400	13.79	0.00	—	13.79	13.30	38.4	1.54
60	1.095	5.11	4.43	0.0373	12.97	1.48	—	12.81	12.41	35.7	1.33
120	1.030	4.77	4.29	0.0351	12.30	2.65	—	12.01	11.31	37.9	1.33
240	0.915	4.54	4.41	0.0311	11.08	4.71	—	10.61	9.98	44.2	1.38
360	0.810	4.49	3.87	0.0276	9.93	6.45	—	9.35	8.64	44.8	1.24
540	0.690	4.28	3.93	0.0235	8.59	8.40	—	7.92	7.31	53.7	1.26
720	0.585	4.21	..	0.0199	7.38	10.04	—	6.69	..	..	..
mean value		4.57	4.34								1.35

$$k \cdot c/x \cdot e \,(\text{sal. f.}) = 1.56 \cdot 10^{-2}$$

Table XL.

Propylglucoside 0.0400 M. Hydrolysis 30°. e = 0.2188 g in 50 ml. sal. f. = 0.044.

1	2	3	4	5	6	7	8	9	10	11	12
0	1.220	—	19.55	0.0400	20.00	0.00	0.00	20.00	19.00	102.9	4.12
20	1.115	19.55	22.55	0.0366	18.60	1.90	1.90	18.00	17.05	132.3	4.84
40	1.005	21.55	23.97	0.0329	17.10	3.80	3.80	16.10	15.15	158.2	5.21
60	0.900	22.02	24.48	0.0295	15.60	5.50	5.50	14.20	12.95	189.0	5.57
90	0.760	22.84	20.42	0.0249	13.40	7.70	7.70	11.70	10.90	187.3	4.66
120	0.660	22.21	21.57	0.0216	11.90	9.30	9.30	10.10	8.80	245.1	5.29
180	0.490	22.01	23.33	0.0160	9.10	11.70	11.70	7.50	6.40	364.5	5.83
240	0.355	22.34	..	0.0117	6.84	13.58	13.58	5.30	..	..	..
mean value		21.8	22.3								5.07

$$k \cdot c/x \cdot e \,(\text{sal. f.}) = 5.27$$

the addition-compounds emulsin-glucoside, emulsin-glucose and emulsin-alcohol.

The temperature coefficient seems to be the same in all cases, the deviations not being greater than may be expected from the inexactness of the measurements. That is to say that the critical increment is the same in all

Table XLI.

Propylglucoside 0.0400 M. Hydrolysis 30°. e = 0.2308 g in
50 ml. sal. f. = 0.044.

1	2	3	4	5	6	7	8	9	10	11	12
0	1.220	—	23.5	0.0400	20.00	0.00	0.00	20.00	18.84	124.6	4.99
20	1.095	23.5	26.3	0.0359	18.32	2.23	2.23	17.67	16.55	159.1	5.71
40	0.970	24.9	23.6	0.0318	16.59	4.36	4.36	15.42	14.54	162.5	5.17
60	0.870	24.6	22.5	0.0285	15.13	6.01	6.01	13.65	12.59	178.3	5.09
90	0.745	23.8	23.1	0.0244	13.25	7.97	7.97	11.52	10.61	218.0	5.33
120	0.635	23.6	29.1	0.0208	11.52	9.64	9.64	9.69	8.01	362.9	7.56
180	0.425	25.4	24.0	0.0139	8.01	12.66	12.66	6.33	5.41	443.9	6.19
240	0.305	25.1	23.6	0.0100	5.88	14.29	14.29	4.48	3.84	615.9	6.16
300	0.220	24.8	..	0.0072	4.31	15.41	15.41	3.20	..	..	..
mean value		24.9	24.9								5.78

$$k \cdot c/x \cdot e \, (\text{sal. f.}) = 5.69 \cdot 10^{-2}$$

Table XLII.

Isopropylglucoside. 0.0400 M. Hydrolysis 30°. e = 0.1845 g
in 50 ml. sal. f. = 0.044.

1	2	3	4	5	6	7	8	9	10	11	12
0	1.245	—	15.3	0.0400	9.09	0.00	0.00	9.09	8.56	178.9	7.16
30	1.120	15.3	12.1	0.0360	8.25	2.17	0.97	8.02	7.65	158.5	5.70
60	1.030	13.7	13.2	0.0331	7.64	3.69	1.66	7.27	6.91	191.6	6.34
90	0.940	13.6	12.9	0.0302	7.02	5.18	2.33	6.54	6.23	206.7	6.24
120	0.860	13.4	10.4	0.0276	6.46	6.45	2.94	5.91	5.48	189.5	5.24
180	0.745	12.4	9.3	0.0239	5.65	8.21	3.78	5.04	4.71	197.9	4.74
240	0.655	11.6	10.7	0.0210	5.00	9.55	4.43	4.37	4.03	265.4	5.59
300	0.565	11.4	8.1	0.0182	4.34	10.80	5.05	3.69	3.50	232.1	4.22
360	0.505	10.9	..	0.0162	3.89	11.68	5.49	3.30	..	..	..
mean value		12.8	11.5								5.65

$$k \cdot c/x \cdot e \, (\text{sal. f.}) = 6.96 \cdot 10^{-2}$$

cases, but that the "constant of action" differs from glu-
coside to glucoside. This constant may be calculated from
the expression

$$\ln k = \frac{-Q}{R \cdot T_1} + \ln \alpha$$

Table XLIII.

Isopropylglucoside. 0.0400 M. Hydrolysis 30°. e = 0.2252 g
in 50 ml. sal. f. = 0.044.

1	2	3	4	5	6	7	8	9	10	11	12
0	1.245	—	16.6	0.0400	9.09	0.00	0.00	9.09	8.51	195.5	7.82
30	1.110	16.6	15.8	0.0357	8.49	2.33	1.04	7.93	7.46	212.3	7.57
60	0.995	16.2	14.5	0.0320	7.40	4.26	1.91	6.99	6.61	219.8	7.03
90	0.900	15.7	15.3	0.0289	6.74	5.81	2.64	6.22	5.88	259.4	7.50
120	0.810	15.6	14.8	0.0260	6.11	7.22	3.30	5.53	4.97	298.2	7.76
180	0.660	15.3	12.5	0.0212	5.03	9.46	4.38	4.41	4.03	311.2	6.60
240	0.555	14.6	12.8	0.0178	4.27	10.98	5.14	3.65	3.34	383.4	6.84
300	0.465	14.3	12.7	0.0149	3.60	12.18	5.77	3.02	2.76	461.3	6.89
360	0.390	14.0	..	0.0125	3.04	13.26	6.29	2.50	..	..	..
mean value	15.3	14.4									7.25

$$k \cdot c/x \cdot e\,(\text{sal. f.}) = 7.32 \cdot 10^{-2}$$

Table XLIV.

Recalculation of table XXXVIII.

1	2 old	2 new	4	5	6	7	9	10	11	12 old	12 new
0	1.175	1.175	6.78	0.0400	13.79	—	13.79	13.14	51.6	2.18	2.06
60	1.065	1.070	6.73	0.0364	12.72	1.96	12.49	11.92	56.5	1.95	2.06
120	0.975	0.975	6.05	0.0332	11.72	3.64	11.35	10.44	57.9	1.78	1.92
240	0.835	0.825	6.21	0.0281	10.00	6.20	9.54	8.76	70.9	2.38	1.99
360	0.690	0.695	4.99	0.0237	8.64	8.30	7.98	7.33	68.0	1.37	1.61
510	0.595	0.585	5.00	0.0199	7.38	10.04	6.69	5.64	88.7	1.93	1.77
840	0.395	0.400	4.84	0.0136	5.22	12.79	4.59	3.45	140.2	1.97	1.91
1440	0.200	0.205	4.51	0.0070	2.72	15.49	2.30	1.99	226.7	1.09	1.58
1740	0.160	0.150	..	0.0051	2.00	16.24	1.68	..	..	..	..
										1.83	1.86

where the integration constant is the constant of action and
Q the critical increment determined by the expression

$$Q = R \cdot \frac{T_1 \cdot T_2}{T_1 - T_2}.$$

In the expression determining α, k has to be calculated
with natural logarithms, whereas the k-values in this paper
are calculated with logarithms to base 10.

The mean value of $\dfrac{k_{30}}{k_{20}}$ is 2.1. From this value is calculated the following values of α:

	Methyl	Ethyl	Propyl	Isopropyl
$\alpha \cdot 10^{-10}$	1.21	1.01	3.16	4.79
K_m	0.62	0.25	0.16	0.40

As the molar concentration of emulsin is not known, the values of α are not the correct ones, but differ from the correct values by a factor which is the same for all glucosides.

An influence of the structure of the aglucone is to be expected on the value of K_m, the dissociation constant of the compound emulsin-glucoside, and of α, the action constant. It is to be seen that the influence on both series of constants is of the same order of magnitude, but that they are not influenced in the same direction.

The examination is continued with glucosides of butyl- and amyl-alcohols.

Summary.

1. The enzymic hydrolysis of propyl- and of isopropyl-β-d-glucoside has been examined. Table XLV gives the values of the velocity-constants, calculated in the usual way (minutes as unit of time, logarithms to base 10), of the affinity constants K_m for the two glucosides, K_{m1} for glucose and K_{m2} for the two alcohols, and of the critical increment Q.

Table XLV.

	Propylglucoside	Isopropylglucoside
$10^2 \cdot k/e \cdot$ (sal. f.) $30°$............	22.6	16.9
» $20°$............	11.3	7.9
K_m $30°$ and $20°$	0.16	0.40
K_{m1} $30°$	0.21	0.15
K_{m2} $30°$	0.18	0.41
Q	12200	13500

2. The fraction of emulsin, bound to the glucoside, is $\dfrac{S}{(K_m + S)}$, S being the glucoside concentration. It has been shown that for the glucosides examined till now, i. e. methyl-, ethyl-, propyl- and isopropyl-glucoside, the product $k\,(K_m + S)$ is independent of the glucoside concentration and has, for the 4 glucosides, the following values:

	Methyl	Ethyl	Propyl	Isopropyl
$10^2 \cdot k \cdot (K_m + S)/e \cdot (\text{sal. f.})$	1.84	1.62	4.33	7.21

3. The fraction of emulsin bound to glucoside, if inhibitors are present, may be calculated when all affinity constants are known.

This fraction x is

$$x = a \cdot \frac{(1-b)\cdot(1-c)}{1 - ab - ac - bc + 2abc}.$$

$\underline{a}$, $\underline{b}$ and $\underline{c}$ being the fractions of emulsin bound to glucoside, glucose and alcohol respectively, if these substances alone were present in the solution.

4. All experiments have been recalculated, the velocity constants being calculated "from point to point". It has been shown that the value of $\dfrac{k \cdot c}{x \cdot e\,(\text{sal. f.})}$, which is identical with k_3 in an equation given by MOELWYN-HUGHES (1936): $k_{obs} = k_3 \cdot \dfrac{[AB]}{[A]\,[B]}$, is constant for all concentrations of glucose and emulsin.

		Methyl	Ethyl	Propyl	Isopropyl
$k_3 = 10^2 \cdot k \cdot c/x \cdot e\,(\text{sal. f.})$	30°	1.89	1.58	5.08	7.51
»	20°	0.94	0.77	2.34	3.62

This means that when for a glucoside the values of k_3, K_m, K_{m1} and K_{m2} and the enzymic power of the emulsin preparation are known, the total description of the hydro-

lysis at any concentration of emulsin, glucoside, glucose and the alcohol in question is possible.

5. The average value of $\dfrac{k_{30}}{k_{20}}$, when the constants are calculated in this way, is 2.1. The critical increment is, therefore, 13100 cal.

6. The action constant, α in the expression $\ln k = \dfrac{-Q}{RT} + \ln \alpha$, for the four glucosides has the following values:

	Methyl	Ethyl	Propyl	Isopropyl
$\alpha \cdot 10^{-10}$	1.21	1.01	3.16	4.79

7. The effect of toluene has been examined in all four cases. The values of $\dfrac{k_{toluene}}{k}$ are

	Methyl	Ethyl	Propyl	Isopropyl
$k_{toluene}/k$	1.55	1.44	1.27	1.08

using emulsin preparations with sal. f.-values about 0.04.

(From the Chemical Laboratory, University of Copenhagen).

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