CCXXXI. STUDIES ON THE ACTION OF EMULSIN. II.

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IN the first paper in this series [Veibel and Bach, 1932] it was qualitatively shown that the difference in the rates of enzymic hydrolysis of the *d*-glucosides of the *d*- and *l*-components of optically active secondary alcohols, which has been examined mainly by Neuberg *et al.* [1929], does not disappear, even if the asymmetric carbon atom is not involved in the glucoside linkage. Thus of the two components of *dl*-3-methylpentyl- β -*d*-glucoside the *d*-alkyl-component is hydrolysed more quickly than the other.

The present paper deals with the extension of the work on the quantitative side and to glucosides prepared from *dl*-1-methylpentanol.

As an indication of the degree of hydrolysis, formerly determined by estimation of the amount of glucose set free, determination of the rotation of the reaction-mixture has been utilised, the enzyme action being stopped by addition of potassium carbonate. The rotations of the emulsin and the glucoside solutions were determined on other samples, and readings were taken to verify that the rotation of these solutions did not change during the experiment. All experiments were carried out at 30° and p_H 4.4.

Before starting the enzymic experiments the ratio of the two components in the glucoside mixture was determined by hydrolysis of the glucoside with acid, the liberated alcohol being isolated and its rotation determined.

Experiments were first made to determine whether the rate of hydrolysis, for a given concentration of glucoside, was proportional to the concentration of emulsin. The rates of hydrolysis of each component separately were determined by a second experiment with nearly the same concentration of glucoside as in the first, but with quantities large enough to permit of samples being withdrawn at different stages of the hydrolysis for examination of the liberated alcohol. From the reaction constants for the *d*- and the *l*-components determined in this way the apparent reaction constants for the first experiments were calculated and were found to correspond fairly well to the constants actually determined. In a further experiment the hydrolysis was allowed to proceed to the equilibrium-point and, in the same way as before, the reaction constants found by experiment were compared with those calculated.

It was found that for the 1-methylpentyl- β -*d*-glucoside the ratio k_d/k_l (k being the unimolecular reaction constant) was 3.44. For 3-methylpentyl- β -*d*-glucoside it was 1.07. In both cases proportionality was found between rate of hydrolysis and concentration of emulsin.

The comparison between experimentally found and calculated reaction constants is based upon an assumption of a linear relation between rotation of the reaction mixture and degree of hydrolysis. For 1-methylpentylglucoside

this does not hold good, owing to the great difference in rates of hydrolysis for the two components. The error involved in assuming a linear relation has been calculated and was found not to exceed 0.005° , *i.e.* the error is within the limit of experiment.

Shortly before my above-mentioned paper [1932] was printed, a very interesting paper by Mitchell and MacArthur [1932] appeared, in which the authors examined the rates of hydrolysis of the glucosides of some optically active alcohols, amongst others 1-methylpentanol. The reaction constants of the two components were determined by exponential analysis, assuming that the curve is of the form

$$y = a_1^{-k_1 t} + a_2^{-k_2 t},$$

where a_1 , a_2 , k_1 , k_2 are constants, t is time and y is proportional to the concentration of unchanged glucoside. The hydrolysis was estimated by determining the amount of glucose set free, and the authors found the ratio $k_2/k_1 = 1.5$. The method employed did not indicate whether the *d*- or the *l*-component were more quickly hydrolysed.

The great difference between this result and the result I have now obtained may be due to several causes. Mitchell and MacArthur employed concentrations, both of glucoside and of emulsin, far lower than those used by me (glucoside $0.0053 M$ against $0.04 M$, emulsin $0.025 g.$ in $50 ml.$ against $0.2 g.$ in $50 ml.$). I have not been able to try whether the concordance is better at such low concentrations, since my method does not allow a reliable control of reactions when the total change of rotation is only about 0.17° . Further, the English authors have possibly worked at a different p_H , since they seem to have dissolved glucoside and emulsin in water without addition of buffer.

Mitchell and MacArthur appear to estimate the ratio between the *d*- and the *l*-components in their glucoside mixture as 1, but they do not state whether they have verified this. They do not indicate the specific rotation of their glucoside tetra-acetate or of their glucoside but give analyses and melting-points only. Now it has been shown by Neuberg and co-workers, especially by von Falkenhausen and Neuberg [1931], in the first place that the synthesis starting from acetobromoglucose may proceed partially asymmetrically, and secondly that during the purification of the tetra-acetate and the glucoside itself by recrystallisation from the solvents commonly used the glucoside mixture may be partially or totally resolved into its components. Unpublished experiments by the author and Frederiksen have shown that the glucoside tetra-acetate of 1-methylpentanol is almost completely resolved by recrystallisation from diluted ethanol, so that a preparation of the acetate with melting-point 99° contains about 80 % *d*- and 20 % *l*-1-methylpentanol in glucosidic linkage, and a preparation of the glucoside with melting-point 115° contains 85–90 % *d*- and 15–10 % *l*-1-methylpentanol. If therefore Mitchell and MacArthur, as seems probable, used a glucoside mixture of 80–90 % *d*- and 20–10 % *l*-1-methylpentyl- β -*d*-glucoside, while estimating the ratio between the two components as 1 in their calculations, their results are decidedly open to question. In their Table II, where the agreement between experiment and calculation is shown, this possible error is however to some degree counterbalanced, since they start their calculations from the time 30 minutes, at which some 25 % of the glucoside has already been hydrolysed. The part first hydrolysed contains naturally most of the component of the highest reaction constant, which at the same time is the one most abundantly present, so that the remaining mixture at the time taken as zero by Mitchell and MacArthur may not differ so much from the mixture of equal parts of the two components as does the starting mixture. Further

examination may show whether this explanation of the difference between our results is correct or not.

For a given sample of emulsin, k/e (k =the unimolecular reaction constant, e =weight of emulsin in 50 ml. reaction mixture) may serve to compare the rates of hydrolysis of different glucosides, when in all cases the same (molar) concentration of glucoside is used. If different samples of emulsin are used, it is necessary to determine the enzymic power of each sample, *e.g.* indicated as sal. f. [Josephson, 1925; Helfferich, 1930; 1931]. In this case k/e (sal. f.) should make possible a comparison of results obtained with different samples of emulsin, but with glucoside solutions of the same molar concentration. In this way it seems possible to compare the influence of different alkyl groups on the rate of hydrolysis. The present investigation is to be continued in this direction.

EXPERIMENTAL.

I. *Standardisation of the emulsin preparation and experimental methods.*

The emulsin preparation was obtained from Merck and was used without further purification. Its enzymic power was determined from its action on salicin [Helfferich, 1930; 1931] and its sal. f. was found to be 0.045.

All experiments were carried out at $30 \pm 0.1^\circ$. The solutions were buffered with an acetate buffer; 100 ml. contained 10.0 ml. 0.1N CH_3COONa and 19.0 ml. 0.1N CH_3COOH ; the p_{H} of such a solution (without glucoside and emulsin) was determined electrometrically at 30° to be 4.4.

The experiments were carried out in the following way.

Solutions of glucoside in water containing acetate buffer and of emulsin in water were placed in a thermostat at 30° . After adjustment of temperature calculated amounts of the two solutions were mixed in a measuring flask, which was then filled to the mark with water at 30° . In order to determine the progress of the hydrolysis samples of 5 ml. were withdrawn at different times and mixed with 1 ml. of 20 % potassium carbonate; after keeping for 3 hours to allow the mutarotation to take place, the rotation of the solution was determined in a 2 dm. tube, if necessary after filtration, and from the difference in rotation the degree of hydrolysis was calculated, the total variation in rotation having first been calculated from the initial rotation of the glucoside solution and the rotation of the resulting solution of glucose and alcohol.

All determinations of rotation were made with a polarimeter from Schmidt and Haensch, Berlin, with triple field, graduation in $\frac{1}{4}^\circ$, vernier scale 0.01° so that 0.005° may be estimated. The source of light was an electric sodium lamp, also from Schmidt and Haensch, and the polarimeter was fitted with a monochromator, allowing the passage of pure D-light only. The polarimeter is a grant from the Carlsberg Foundation, to which my thanks are due.

II. *1-Methylpentyl- β -D-glucoside.*

(a) *Preparation and description.* The tetra-acetyl compound was prepared in the usual manner from acetobromoglucose, 1-methylpentanol and silver carbonate. The acetyl groups were removed, either with baryta as indicated by Fischer and Raske [1909], or with sodium methoxide according to Zemplén and Pascu [1929]. The two methods gave approximately the same yields.

The preparation here used was a mixture of the two glucosides corresponding to the *d*- and the *l*-components of the alcohol. The melting-point was, even after several recrystallisations from ethyl acetate, rather indefinite, 60 – 67° . The specific rotation of the preparation, which was used for all experiments

here recorded, was $[\alpha]_D^{19} = -32.50^\circ$ (water: $c=3.323$, $l=2$). (Found: C, 54.84; H, 8.95 %. $C_{12}H_{24}O_6$ requires C, 54.50; H, 9.16 %.)

3 g. of the glucoside were boiled for 2 hours with 200 ml. water and 25 ml. 4*N* sulphuric acid. The alcohol set free was then distilled off with the first 100 ml. distillate. The distillate was extracted with ether, the ethereal solution dried over potassium carbonate, the ether distilled off and the residue fractionated. The fraction with b.p. 135–40° was regarded as pure 1-methylpentanol. Its rotation in a 1 dm. tube was $+1.05^\circ$; the density being 0.823, $[\alpha]_D^{19}$ is found to be $+1.28^\circ$.

Pickard and Kenyon [1911] give for the optically pure compound $[\alpha]_D = \pm 11.57^\circ$; a preparation with $[\alpha]_D = +1.28^\circ$ contains therefore 55.5 % *d*- and 44.5 % *l*-compound.

As mentioned above, the glucoside tetra-acetate is partially resolved into its two components by recrystallisation from dilute alcohol. The examination of the degree of this resolution is being continued.

(b) *Proportionality between concentration of emulsin and rate of hydrolysis.*

A solution of glucoside in buffer solution was prepared which contained 2.6278 g. glucoside in 100 ml., and a solution of emulsin in water which contained 0.0719 g. in 10 ml. The emulsin solution was made by shaking 1 g. of emulsin thoroughly with water, filtering the mixture and filling up to 100 ml. in a measuring flask. The solid content of the solution was determined by evaporating 10 ml. to dryness at 105°.

Mixtures as indicated in Tables I–V were then prepared in measuring flasks of 50 ml. and samples of 5 ml. were withdrawn at times stated in the tables. The samples were mixed with 1 ml. 20 % potassium carbonate and their rotations determined. The emulsin solution had a rotation of -0.13° during the whole of the experiment, the glucoside solution a rotation of -0.57° . From this value the rotation of the solution after complete hydrolysis is calculated to $+0.64^\circ$, taking into account that the 1-methylpentanol present has a specific rotation of $+1.28^\circ$.

Table I.

10 ml. emulsin solution, water + buffer to 50 ml.

$c_{\text{emulsin}} = 0.0719$ g. in 50 ml.,
 $c_{\text{glucoside}} = 0$.

Time min.	α
5	-0.13
50	-0.13
100	-0.14
1500	-0.13

Table II.

20 ml. glucoside solution, water to 50 ml.

$c_{\text{emulsin}} = 0$,
 $c_{\text{glucoside}} = 0.0398$ *M*.

Time min.	α
5	-0.56
50	-0.57
100	-0.57
1500	-0.57

Table III.

10 ml. emulsin solution, 20 ml. glucoside solution, water to 50 ml.,

$c_{\text{emulsin}} = 0.0719$ g. in 50 ml., $c_{\text{glucoside}} = 0.0398$ *M*.

Time min.	α	$\alpha_{\text{gluc.}}$	x	$c - x$	$k \cdot 10^4$	%
0-00	-0.70	-0.57	—	1.21	—	—
9-97	-0.58	-0.45	0.12	1.09	45.5	9.9
20-00	-0.49	-0.36	0.21	1.00	41.4	17.4
40-00	-0.31	-0.18	0.39	0.82	42.3	32.2
60-00	-0.18	-0.05	0.52	0.69	40.7	43.0
80-00	-0.08	$+0.05$	0.62	0.59	39.0	51.2
110-00	$+0.05$	$+0.18$	0.75	0.46	38.2	62.0
150-00	$+0.15$	$+0.28$	0.85	0.36	35.1	70.2

Table IV.

20 ml. emulsin solution, 20 ml. glucoside solution, water to 50 ml.

 $c_{\text{emulsin}} = 0.1438 \text{ g. in 50 ml.}$, $c_{\text{glucoside}} = 0.0398 \text{ M.}$

Time min.	α	$\alpha_{\text{gluc.}}$	x	$c - x$	$k \cdot 10^4$	%
0-00	-0.83	-0.57	—	1.21	—	—
10-00	-0.62	-0.36	0.21	1.00	82.8	17.4
20-00	-0.46	-0.20	0.37	0.84	79.3	30.8
39.97	-0.22	+0.04	0.61	0.60	76.2	50.4
60-00	-0.06	+0.20	0.77	0.44	73.2	63.6
80-00	+0.04	+0.30	0.87	0.34	68.9	71.9
110-00	+0.13	+0.39	0.96	0.25	62.3	79.2
150-00	+0.20	+0.46	1.03	0.18	55.2	85.1

Table V.

30 ml. emulsin solution, 20 ml. glucoside solution.

 $c_{\text{emulsin}} = 0.2157 \text{ g. in 50 ml.}$, $c_{\text{glucoside}} = 0.0398 \text{ M.}$

Time min.	α	$\alpha_{\text{gluc.}}$	x	$c - x$	$k \cdot 10^4$	%
0-00	-0.96	-0.57	—	1.21	—	—
10-00	-0.66	-0.27	0.30	0.91	123.8	24.8
20-00	-0.44	-0.05	0.52	0.69	122.0	43.0
40-00	-0.18	+0.21	0.78	0.43	112.3	64.5
60-00	-0.04	+0.35	0.92	0.29	103.4	76.1
80-00	+0.06	+0.45	1.02	0.19	100.5	84.3
110-00	+0.12	+0.51	1.08	0.13	88.1	89.3
150-00	+0.16	+0.55	1.12	0.09	75.2	92.6

From the tables it is seen that the unimolecular reaction constants (which are all calculated with logarithms to base 10 and the minute as unit of time) fall steadily at all concentrations of emulsin. This may indicate that the two components of the glucoside are hydrolysed with rather different velocities.

The initial values of the constants show that there is a fairly good proportionality between concentration of emulsin and velocity of hydrolysis, the value of k/e being about $5.8 \cdot 10^{-2}$.

(c) *Determination of the rates of hydrolysis of the two components.* In order to find the reaction constants of the two components the following experiment was made:

15 g. glucoside were dissolved in 600 ml. acetate-buffer and mixed with 900 ml. emulsin solution, both solutions having been brought to 30° . The concentration of glucoside in the resulting mixture was 0.0391 M , the rotation of the glucoside was -0.560° at the beginning, $+0.625^\circ$ after complete hydrolysis. The concentration of the emulsin solution was $0.2067 \text{ g. in 50 ml.}$, the rotation of the emulsin present in the solution was -0.375° . The velocity constant was approximately calculated from the relation $k/e = 5.8 \cdot 10^{-2}$ and the times necessary to bring about a hydrolysis of 25 %, 40 % and 65 % respectively were calculated. At times determined in this way samples of 5 ml. were withdrawn and treated as usual, and 1 minute after each sample of 5 ml. samples large enough to contain about 2 g. hydrolysed glucoside were taken; the enzyme action in these samples was stopped by addition of a concentrated potassium carbonate solution, the alcohol set free was isolated in the way described above, collected in 5 ml. measuring flasks and filled up to the mark with absolute alcohol, and then the rotation was determined. The specific rotation of the optically pure

component, which is 11.57° without solvent, was determined in absolute alcoholic solution and found to be 12.48° ($c=10.328$, $l=2$). Consequently the percentage content of d -component in a certain solution is calculated from the equation

$$12.48.d - 12.48(100-d) = \alpha.100,$$

α being the experimentally found specific rotation.

The real degree of hydrolysis of the large samples is calculated from the foregoing 5 ml. sample, assuming that the reaction constant does not alter sensibly during 1 minute.

The results are given in Tables VI and VII. The figures in heavy type in Table VII are the calculated figures.

Table VI.

Rotation of the alcohol isolated.

10.95 min. $\alpha^{15^\circ} = +1.12^\circ$, $c=7.756$, $l=2$, $[\alpha]_D^{15^\circ} = +7.22^\circ$.

22.00 min. $\alpha^{16^\circ} = +0.87^\circ$, $c=6.780$, $l=2$, $[\alpha]_D^{16^\circ} = +6.62^\circ$.

45.00 min. $\alpha^{15^\circ} = +1.09^\circ$, $c=9.730$, $l=2$, $[\alpha]_D^{15^\circ} = +5.60^\circ$.

Table VII.

$c_{\text{emulsin}} = 0.2067$ g. in 50 ml., $c_{\text{glucoside}} = 0.0391$ M.

Mixed Sample	5 ml.	Time min.	α	$\alpha_{\text{gluc.}}$	x	$c-x$	$k \cdot 10^4$	%	Containing	
									% d	% l
		0.00	-0.935	-0.560	—	1.185	—	—	—	—
		10.00	-0.650	-0.275	0.285	0.900	119.5	24.1	—	—
	710	10.95	—	—	0.308	0.877	119.5	26.0	78.9	21.1
	5	21.00	-0.445	-0.070	0.490	0.695	110.4	41.4	—	—
	460	22.00	—	—	0.508	0.877	110.4	42.9	75.7	24.3
	5	44.00	-0.190	+0.185	0.745	0.440	97.8	62.9	—	—
	310	45.00	—	—	0.755	0.430	97.8	63.7	72.4	27.6

It is now possible to calculate the concentration of either component at the time at which the samples were taken and thus to calculate the two velocity constants. This is done in Table VIII.

Table VIII.

Time min.	x_d	x_l	$c_d - x_d$	$c_l - x_l$	$k_d \cdot 10^4$	$k_l \cdot 10^4$	k_d/k_l
0	—	—	0.0217	0.0174	—	—	—
10.95	0.0080	0.0021	0.0137	0.0153	182.4	51.0	3.46
22.00	0.0127	0.0041	0.0090	0.0133	173.7	53.1	3.31
45.00	0.0180	0.0069	0.0037	0.0105	170.7	48.8	3.55
Mean values					175.6	51.0	3.44

In order to use these constants for controlling experiments with other emulsin concentrations it is necessary to calculate the quotients k/e , e being the emulsin concentration indicated as g. emulsin in 50 ml. reaction mixture, here 0.2067.

We thus get $k_d/e = 8.50 \cdot 10^{-2}$, $k_l/e = 2.47 \cdot 10^{-2}$.

In Tables IX–XI the experiments described above in part (b) are controlled in this way. It may be seen that the values calculated agree tolerably well with those found, the deviation in α being only in one case as much as 0.03° , in most cases 0.01° or less.

Table IX.

$$c_d = 0.0221, k_d = 61.1 \cdot 10^{-4}, c_l = 0.0177, k_l = 17.9 \cdot 10^{-4}, e = 0.0719.$$

Time min.	$c_d - x_d$	x_d	$c_l - x_l$	x_l	x_{tot}	$c - x_{tot}$	$k \cdot 10^4$		%		α	
							calc.	f.	calc.	f.	calc.	f.
0	0.0221	—	0.0177	—	—	0.0398	—	—	—	—	-0.57	-0.57
10	0.0192	0.0029	0.0170	0.0007	0.0036	0.0362	41.2	45.5	9.1	9.9	-0.46	-0.45
20	0.0167	0.0054	0.0163	0.0014	0.0068	0.0330	40.7	41.4	17.1	17.4	-0.36	-0.36
40	0.0126	0.0095	0.0150	0.0027	0.0122	0.0276	39.7	42.3	30.7	32.2	-0.20	-0.18
60	0.0095	0.0126	0.0138	0.0039	0.0165	0.0233	38.8	40.7	41.5	43.0	-0.07	-0.05
80	0.0072	0.0149	0.0127	0.0050	0.0199	0.0199	37.6	39.0	50.0	51.2	+0.04	+0.05
110	0.0047	0.0174	0.0112	0.0065	0.0239	0.0159	36.2	38.2	60.1	62.0	+0.16	+0.18
150	0.0027	0.0194	0.0095	0.0082	0.0276	0.0122	34.2	35.1	69.4	70.2	+0.27	+0.28

Table X.

$$c_d = 0.0221, k_d = 122.2 \cdot 10^{-4}, c_l = 0.0177, k_l = 35.7 \cdot 10^{-4}, e = 0.1438.$$

Time min.	$c_d - x_d$	x_d	$c_l - x_l$	x_l	x_{tot}	$c - x_{tot}$	$k \cdot 10^4$		%		α	
							calc.	f.	calc.	f.	calc.	f.
0	0.0221	—	0.0177	—	—	0.0398	—	—	—	—	-0.57	-0.57
10	0.0167	0.0054	0.0163	0.0014	0.0068	0.0330	81.4	82.8	17.1	17.4	-0.36	-0.36
20	0.0126	0.0095	0.0150	0.0027	0.0122	0.0276	79.5	79.3	30.7	30.6	-0.20	-0.20
40	0.0072	0.0149	0.0127	0.0050	0.0199	0.0199	75.3	76.2	50.0	50.4	+0.04	+0.04
60	0.0041	0.0180	0.0108	0.0069	0.0249	0.0149	71.1	73.2	62.6	63.6	+0.19	+0.20
80	0.0023	0.0198	0.0092	0.0085	0.0283	0.0115	67.4	68.9	71.1	71.9	+0.29	+0.30
110	0.0010	0.0211	0.0072	0.0105	0.0316	0.0082	62.4	62.3	79.4	79.2	+0.39	+0.39
150	0.0003	0.0218	0.0052	0.0125	0.0343	0.0055	57.3	55.2	86.2	85.1	+0.47	+0.46

Table XI.

$$c_d = 0.0221, k_d = 183.4 \cdot 10^{-4}, c_l = 0.0177, k_l = 53.6 \cdot 10^{-4}, e = 0.2157.$$

Time min.	$c_d - x_d$	x_d	$c_l - x_l$	x_l	x_{tot}	$c - x_{tot}$	$k \cdot 10^4$		%		α	
							calc.	f.	calc.	f.	calc.	f.
0	0.0221	—	0.0177	—	—	0.0398	—	—	—	—	-0.57	-0.57
10	0.0145	0.0076	0.0156	0.0021	0.0097	0.0301	121.3	123.8	24.4	24.8	-0.27	-0.27
20	0.0095	0.0126	0.0138	0.0039	0.0165	0.0233	116.3	122.0	41.5	43.0	-0.07	-0.05
40	0.0041	0.0180	0.0108	0.0069	0.0249	0.0149	106.7	112.3	62.6	64.5	+0.19	+0.21
60	0.0018	0.0203	0.0084	0.0093	0.0296	0.0102	98.6	103.4	74.4	76.1	+0.33	+0.35
80	0.0008	0.0213	0.0066	0.0111	0.0324	0.0074	91.3	100.5	81.4	84.3	+0.42	+0.45
110	0.0002	0.0219	0.0046	0.0131	0.0350	0.0048	83.5	88.1	87.9	89.3	+0.49	+0.51
150	0.0000	0.0221	0.0028	0.0149	0.0370	0.0028	76.9	75.2	93.0	92.6	+0.56	+0.55

(d) *Determination of the equilibrium-point.* In an experiment with approximately the same emulsin and glucoside concentrations as in part (c) the hydrolysis was followed during 480 minutes. An additional reading was made after 1440 minutes. Table XII gives the result, which is that the equilibrium-point is at some 95 % hydrolysis.

Table XIII gives a comparison of the calculated and the experimentally found values of k , degree of hydrolysis and α . The calculation is made in the same way as in Tables IX–XI. $c_{\text{glucoside}}$ is here $0.0406 M$, c_{emulsin} is $0.2006 g.$ in $50 ml.$, k_d is calculated to be $170.5 \cdot 10^{-4}$, k_l to be $49.5 \cdot 10^{-4}$.

A calculation of α like the one here given is based upon the assumption of a linear relation between rotation of the reaction mixture and degree of hydrolysis. As the relation k_d/k_l has been found to be 3.44, this assumption does not hold good. In order to make an exact calculation it is necessary to know the specific rotations of the two glucoside components. These rotations are not yet known, but unpublished experiments (by the author and Frederiksen) make it seem probable that they are not far from -29.5° and -36.7° respectively. Since the difference between the figures calculated with these values as base

Table XII.

 $c_{\text{emulsin}} = 0.2006 \text{ g. in } 50 \text{ ml.}, \alpha_{\text{emulsin}} = -0.365^\circ.$ $c_{\text{glucoside}} = 0.0406 \text{ M}, \alpha_{\text{beginning}} = -0.580^\circ, \alpha_{\text{end}} = +0.645^\circ.$

Time min.	α	$\alpha_{\text{corr.}}$	x	$c - x$	$k \cdot 10^4$	%
0.0	-0.945	-0.580	—	1.225	—	—
5.0	-0.800	-0.435	0.145	1.080	109.4	11.8
10.0	-0.670	-0.305	0.275	0.950	110.5	22.4
20.0	-0.480	-0.115	0.465	0.760	103.7	38.0
40.0	-0.215	+0.150	0.730	0.495	98.4	59.6
60.0	-0.085	+0.280	0.860	0.365	87.6	70.2
80.0	+0.020	+0.385	0.965	0.260	84.2	78.8
100.0	+0.070	+0.435	1.015	0.210	76.6	82.9
120.0	+0.105	+0.470	1.050	0.175	70.4	85.7
140.0	+0.135	+0.500	1.080	0.145	66.2	88.2
160.0	+0.175	+0.540	1.120	0.105	66.7	91.4
190.0	+0.195	+0.560	1.140	0.085	61.0	93.1
220.0	+0.185	+0.550	1.130	0.095	—	92.2
250.0	+0.200	+0.565	1.145	0.080	—	93.5
280.0	+0.200	+0.565	1.145	0.080	—	93.5
310.0	+0.200	+0.565	1.145	0.080	—	93.5
360.0	+0.205	+0.570	1.150	0.075	—	93.9
420.0	+0.210	+0.575	1.155	0.070	—	94.3
480.0	+0.220	+0.585	1.165	0.060	—	95.1
1440.0	+0.220	+0.585	1.165	0.060	—	95.1

Table XIII.

 $c_d = 0.0225, k_d = 170.5 \cdot 10^{-4}, c_l = 0.0181, k_l = 49.5 \cdot 10^{-4}, e = 0.2006.$

Time min.	$c_d - x_d$	x_d	$c_l - x_l$	x_l	x_{tot}	$c - x_{\text{tot}}$	$k \cdot 10^4$		%		α	
							calc.	f.	calc.	f.	calc.	f.
0	0.0225	—	0.0181	—	—	0.0406	—	—	—	—	-0.580	-0.580
5	0.0185	0.0040	0.0171	0.0010	0.0050	0.0356	114.2	109.4	12.3	11.8	-0.430	-0.435
10	0.0152	0.0073	0.0162	0.0019	0.0092	0.0314	111.6	110.5	22.7	22.4	-0.300	-0.305
20	0.0103	0.0122	0.0144	0.0037	0.0159	0.0247	107.9	103.7	39.2	38.0	-0.100	-0.115
40	0.0047	0.0178	0.0115	0.0066	0.0244	0.0162	99.8	98.4	60.1	59.6	+0.155	+0.150
60	0.0021	0.0204	0.0091	0.0090	0.0294	0.0112	93.2	87.6	72.4	70.2	+0.305	+0.280
80	0.0010	0.0215	0.0073	0.0108	0.0323	0.0083	86.2	84.2	79.6	78.8	+0.395	+0.385
100	0.0004	0.0221	0.0058	0.0123	0.0344	0.0062	81.6	76.6	84.7	82.9	+0.460	+0.435
120	0.0002	0.0223	0.0046	0.0135	0.0358	0.0048	77.3	70.4	88.2	85.7	+0.500	+0.470
140	0.0001	0.0224	0.0037	0.0144	0.0368	0.0038	73.5	66.2	90.6	88.2	+0.530	+0.500
160	0.0000	0.0225	0.0029	0.0152	0.0377	0.0029	71.6	66.7	92.9	91.4	+0.555	+0.540
190	0.0000	0.0225	0.0021	0.0160	0.0385	0.0021	67.7	61.0	94.8	93.1	+0.580	+0.560

and those calculated assuming a linear relation does not exceed 0.005° , which is within the experimental limits, I have given the "linear" figures only.

It will be seen from Table XIII that up to some 70 % hydrolysis the agreement between the calculated and the found values of α is excellent. The agreement between calculated and found values of k and degree of hydrolysis is not quite as good; this is due to the fact that the found values are calculated from the found values of α and, owing to the deviation from a linear relation mentioned above, the real degree of hydrolysis is somewhat greater than that calculated from α .

III. 3-Methylpentyl- β -D-glucoside.

(a) *Preparation and description.* The glucoside was prepared as previously described [Veibel and Bach, 1932], the acetyl groups however being removed as indicated by Zemplén and Pascu [1929]. (Found: C, 54.20; H, 9.16 %. $\text{C}_{12}\text{H}_{24}\text{O}_2$ requires C, 54.50; H, 9.16 %.) The melting-point was $83-88^\circ$, the specific rotation $-30-85^\circ$ (water, $c = 2.269$, $l = 2$).

2.5 g. glucoside were unspecifically hydrolysed and yielded 0.3304 g. alcohol with B.P. 145–51°, which, dissolved in absolute alcohol to 5 ml., gave a rotation of -0.015° , whence $[\alpha]_D = -0.111^\circ$. $[\alpha]_D$ for the optically pure compound being $+8.77^\circ$ [Hardin, 1908], this means that the content of *d*-component is 49.37 %, of *l*-component 50.63 %.

(b) *Proportionality between concentration of emulsin and rate of hydrolysis.*

The experiments were made in exactly the same manner as indicated above for the 1-methylpentylglucoside. The reaction mixtures had the composition indicated in Tables XIV–XVIII. The emulsin solution had a rotation of -0.13° , the glucoside solution a rotation of -0.540° . From the latter value the rotation of the solution after complete hydrolysis is calculated to be $+0.625^\circ$, the rotation of the alcohol set free being without measurable importance.

It will be seen that, apart from the initial values, the unimolecular reaction constants are real constants, and that there is sufficient evidence for the proportionality between the concentration of emulsin and the rate of hydrolysis,

Table XIV.

10 ml. emulsin solution, water + buffer to 50 ml.

$c_{\text{emulsin}} = 0.0712$ g. in 50 ml.,
 $c_{\text{glucoside}} = 0.$

Time min.	α
0	-0.130
160	-0.130
1620	-0.130

Table XV.

20 ml. glucoside solution, water to 50 ml.

$c_{\text{emulsin}} = 0.$
 $c_{\text{glucoside}} = 0.0398$ M.

Time min.	α
0	-0.540
160	-0.540
1620	-0.540

Table XVI.

10 ml. emulsin solution, 20 ml. glucoside solution, water to 50 ml.

$c_{\text{emulsin}} = 0.0712$ g. in 50 ml., $c_{\text{glucoside}} = 0.0398$ M.

Time min.	α	$\alpha_{\text{gluc.}}$	x	$c - x$	$k \cdot 10^4$	%
0-00	-0.670	-0.540	—	1.165	—	—
5-15	-0.660	-0.530	0.010	1.155	7.3	0.9
10-00	-0.645	-0.515	0.025	1.140	9.4	2.2
20-00	-0.630	-0.500	0.040	1.125	7.6	3.4
40-00	-0.600	-0.470	0.070	1.095	6.7	6.0
60-00	-0.570	-0.440	0.100	1.065	6.5	8.6
80-00	-0.550	-0.420	0.120	1.045	5.9	10.3
110-00	-0.485	-0.355	0.185	0.980	6.8	15.9
150-00	-0.450	-0.320	0.220	0.945	6.1	18.9
1620-00	$+0.395$	$+0.525$	1.065	0.100	6.6	91.4

Table XVII.

20 ml. emulsin solution, 20 ml. glucoside solution, water to 50 ml.

$c_{\text{emulsin}} = 0.1424$ g. in 50 ml., $c_{\text{glucoside}} = 0.0398$ M.

Time min.	α	$\alpha_{\text{gluc.}}$	x	$c - x$	$k \cdot 10^4$	%
0-00	-0.800	-0.540	—	1.165	—	—
5-00	-0.775	-0.515	0.025	1.140	18.9	2.2
10-00	-0.745	-0.485	0.055	1.110	21.0	4.7
20-00	-0.715	-0.455	0.085	1.080	16.5	7.3
40-00	-0.660	-0.400	0.140	1.025	13.9	12.0
60-00	-0.610	-0.350	0.190	0.975	12.9	16.3
80-00	-0.540	-0.280	0.260	0.905	13.7	22.3
110-00	-0.480	-0.220	0.320	0.845	12.7	27.5
150-00	-0.400	-0.140	0.400	0.765	12.2	34.3
1620-00	$+0.320$	$+0.580$	1.120	0.045	—	96.1

Table XVIII.

30 ml. emulsin solution, 20 ml. glucoside solution.
 $c_{\text{emulsin}} = 0.2136 \text{ g. in } 50 \text{ ml.}$, $c_{\text{glucoside}} = 0.0398 M$.

Time min.	α	$\alpha_{\text{gluc.}}$	x	$c - x$	$k \cdot 10^4$	%
0-00	-0.930	-0.540	—	1.165	—	—
5-00	-0.875	-0.485	0.055	1.110	42.0	4.7
10-00	-0.850	-0.460	0.080	1.085	30.9	6.9
20-00	-0.805	-0.415	0.125	1.040	24.7	10.7
40-00	-0.720	-0.330	0.210	0.955	21.6	18.0
60-00	-0.650	-0.260	0.280	0.885	19.9	24.0
80-00	-0.575	-0.185	0.355	0.810	19.7	30.5
110-00	-0.500	-0.110	0.430	0.735	18.2	36.9
150-00	-0.380	+0.010	0.550	0.615	18.5	47.2
1620-00	+0.200	+0.590	1.130	0.035	—	97.0

which is in this case only 1/6 to 1/7 of the rate of hydrolysis of 1-methylpentyl-glucoside, k/e being only about $0.9 \cdot 10^{-2}$ against $5.8 \cdot 10^{-2}$.

(c) *Determination of the rates of hydrolysis of the two components.* From the constancy of the reaction constants determined above it seems likely that the two components of the glucoside are hydrolysed with nearly the same velocity. In order to determine the two reaction constants the following experiment was made.

10 g. glucoside were dissolved in 500 ml. acetate buffer and mixed with 500 ml. emulsin solution, both solutions having been brought to 30° . The concentration of glucoside in the resulting mixture was $0.0398 M$, the rotation of the glucoside was -0.540° at the beginning, $+0.625^\circ$ after complete hydrolysis. The concentration of emulsin was $0.1829 \text{ g. in } 50 \text{ ml.}$, the rotation of the emulsin was -0.325° . The velocity constant was determined by interpolation to be approximately $16 \cdot 10^{-4}$, and the times necessary for bringing about a hydrolysis of 40 % and 60 % respectively were calculated. As in the foregoing experiment small samples were taken in order to control the degree of hydrolysis and larger samples to isolate the alcohol set free in order to determine how much of each component it contained. Tables XIX and XX give the results.

Table XIX.

Rotation of the alcohol isolated.

136 min. $\alpha^{19^\circ} = +0.015^\circ$, $c = 6.418$, $l = 2$, $[\alpha]_D^{19^\circ} = +0.117^\circ$.

243 min. $\alpha^{19^\circ} = \pm 0^\circ$, $[\alpha]_D^{19^\circ} = \pm 0^\circ$.

Table XX.

$c_{\text{emulsin}} = 0.1829 \text{ g. in } 50 \text{ ml.}$, $c_{\text{glucoside}} = 0.0398 M$.

		Time min.	α	$\alpha_{\text{gluc.}}$	x	$c - x$	$k \cdot 10^4$	%	Containing	
									% d	% l
Mixed		0	-0.865	-0.540	—	1.165	—	—	—	—
Sample	5 ml.	135	-0.420	-0.095	0.445	0.720	15.45	38.20	—	—
"	590 "	136	—	—	0.447	0.718	15.45	38.37	50.67	49.33
"	5 "	242	-0.200	+0.125	0.665	0.500	15.18	57.08	—	—
"	390 "	243	—	—	0.667	0.498	15.18	57.25	50.00	50.00

The calculated values of the concentration of either component and of k_d and k_l are given in Table XXI.

Table XXI.

Time min.	$c_d - x_d$	x_d	$c_l - x_l$	x_l	$k_d \cdot 10^4$	$k_l \cdot 10^4$	k_d/k_l
0	0.0196	—	0.0202	—	—	—	—
136	0.0118	0.0078	0.0127	0.0075	16.2	14.8	1.09
243	0.0082	0.0114	0.0088	0.0114	15.6	14.8	1.05
Mean values					15.9	14.8	1.07

e being 0.1829, we get $k_d/e = 0.869 \cdot 10^{-2}$, $k_l/e = 0.809 \cdot 10^{-2}$. With these values k_d and k_l in the experiments described in Tables XVI–XVIII are calculated and the found values of k , degree of hydrolysis and α are compared with those calculated.

Table XXII.

$$c_d = 0.0196, k_d = 6.2 \cdot 10^{-4}, c_l = 0.0202, k_l = 5.8 \cdot 10^{-4}, e = 0.0712.$$

Time min.	$c_d - x_d$	x_d	$c_l - x_l$	x_l	$x_{tot.}$	$c - x_{tot.}$	$k \cdot 10^4$		%		α	
							calc.	f.	calc.	f.	calc.	f.
0	0.0196	—	0.0202	—	—	0.0398	—	—	—	—	—0.540	—0.540
5	0.0195	0.0001	0.0201	0.0001	0.0002	0.0396	4.2	7.3	0.5	0.9	—0.535	—0.530
10	0.0193	0.0003	0.0199	0.0003	0.0006	0.0392	6.6	9.4	1.5	2.2	—0.520	—0.515
20	0.0190	0.0006	0.0197	0.0005	0.0011	0.0387	6.1	7.6	2.8	3.4	—0.505	—0.500
40	0.0185	0.0011	0.0191	0.0011	0.0022	0.0376	6.2	6.7	5.5	6.0	—0.475	—0.470
60	0.0180	0.0016	0.0186	0.0016	0.0032	0.0366	6.1	6.5	8.0	8.6	—0.445	—0.440
80	0.0175	0.0021	0.0182	0.0020	0.0041	0.0357	5.9	5.9	10.3	10.3	—0.420	—0.420
110	0.0168	0.0028	0.0174	0.0028	0.0056	0.0342	6.0	6.8	14.1	15.9	—0.375	—0.355
150	0.0158	0.0038	0.0165	0.0037	0.0075	0.0323	6.0	6.1	18.8	18.9	—0.320	—0.320

Table XXIII.

$$c_d = 0.0196, k_d = 12.4 \cdot 10^{-4}, c_l = 0.0202, k_l = 11.5 \cdot 10^{-4}, e = 0.1424.$$

Time min.	$c_d - x_d$	x_d	$c_l - x_l$	x_l	$x_{tot.}$	$c - x_{tot.}$	$k \cdot 10^4$		%		α	
							calc.	f.	calc.	f.	calc.	f.
0	0.0196	—	0.0202	—	—	0.0398	—	—	—	—	—0.540	—0.540
5	0.0193	0.0003	0.0199	0.0003	0.0006	0.0392	13.2	18.9	1.5	2.2	—0.520	—0.515
10	0.0190	0.0006	0.0197	0.0005	0.0011	0.0387	12.2	21.0	2.8	4.7	—0.505	—0.485
20	0.0185	0.0011	0.0192	0.0010	0.0021	0.0377	11.8	16.5	5.3	7.3	—0.480	—0.455
40	0.0175	0.0021	0.0182	0.0020	0.0041	0.0357	11.8	13.9	10.3	12.0	—0.420	—0.400
60	0.0165	0.0031	0.0172	0.0030	0.0061	0.0337	12.0	12.9	15.3	16.3	—0.360	—0.350
80	0.0156	0.0040	0.0163	0.0039	0.0079	0.0319	12.0	13.7	19.8	22.3	—0.310	—0.280
110	0.0143	0.0053	0.0151	0.0051	0.0104	0.0294	12.0	12.7	26.1	27.5	—0.235	—0.220
150	0.0128	0.0068	0.0136	0.0066	0.0134	0.0264	11.9	12.2	33.7	34.3	—0.145	—0.145

Table XXIV.

$$c_d = 0.0196, k_d = 18.6 \cdot 10^{-4}, c_l = 0.0202, k_l = 17.3 \cdot 10^{-4}, e = 0.2136.$$

Time min.	$c_d - x_d$	x_d	$c_l - x_l$	x_l	$x_{tot.}$	$c - x_{tot.}$	$k \cdot 10^4$		%		α	
							calc.	f.	calc.	f.	calc.	f.
0	0.0196	—	0.0202	—	—	0.0396	—	—	—	—	—0.540	—0.540
5	0.0192	0.0004	0.0198	0.0004	0.0008	0.0390	17.6	42.0	2.0	4.7	—0.515	—0.485
10	0.0188	0.0008	0.0194	0.0008	0.0016	0.0382	17.8	30.9	4.0	6.9	—0.495	—0.460
20	0.0180	0.0016	0.0187	0.0015	0.0031	0.0367	17.6	24.7	7.8	10.7	—0.450	—0.415
40	0.0165	0.0031	0.0172	0.0030	0.0061	0.0337	18.1	21.6	15.3	18.0	—0.360	—0.330
60	0.0152	0.0044	0.0159	0.0043	0.0087	0.0311	17.9	19.9	21.9	24.0	—0.285	—0.260
80	0.0139	0.0057	0.0147	0.0055	0.0112	0.0286	17.9	19.7	28.1	30.5	—0.210	—0.185
110	0.0122	0.0074	0.0130	0.0072	0.0146	0.0252	18.0	18.2	36.7	36.9	—0.110	—0.110
150	0.0103	0.0093	0.0111	0.0091	0.0184	0.0214	18.0	18.5	46.2	47.2	—0.000	+0.010

Here the agreement is not quite as good as in the case of the 1-methylpentyl-glucoside. The initial values differ especially. It may be that the value for e in these experiments is a little too low; if this is the case the deviations between calculated and found values will certainly be maximum in the experiment with highest emulsin concentration.

(d) *Determination of the equilibrium-point.* In this experiment the hydrolysis was followed during 720 minutes and an additional reading was taken after 1470 minutes. Table XXV gives the result, which is that the equilibrium-point is at some 96 % hydrolysis.

Table XXV.

$c_{\text{emulsin}} = 0.1896 \text{ g. in } 50 \text{ ml.}, \alpha_{\text{emulsin}} = -0.335^\circ.$
 $c_{\text{glucoside}} = 0.0398 \text{ M}, \alpha_{\text{beg.}} = -0.540^\circ, \alpha_{\text{end}} = +0.625^\circ.$

Time min.	α	$\alpha_{\text{gluc.}}$	x	$c-x$	$k \cdot 10^4$	%
0.0	-0.875	-0.540	—	1.165	—	—
1.7	-0.865	-0.530	0.010	1.155	22.1	0.9
10.0	-0.815	-0.480	0.060	1.105	23.0	5.2
30.0	-0.740	-0.405	0.135	1.030	17.8	11.6
60.0	-0.640	-0.305	0.235	0.930	16.3	20.2
90.0	-0.530	-0.195	0.345	0.820	16.9	29.6
120.0	-0.450	-0.115	0.425	0.740	16.4	36.5
150.0	-0.375	-0.040	0.500	0.665	16.2	42.9
180.0	-0.305	+0.030	0.570	0.595	16.2	48.9
210.0	-0.240	+0.095	0.635	0.530	16.3	54.5
240.0	-0.190	+0.145	0.685	0.480	16.0	58.8
270.0	-0.140	+0.195	0.735	0.430	16.0	63.1
300.0	-0.090	+0.245	0.785	0.380	16.2	67.4
360.0	-0.010	+0.325	0.865	0.300	16.4	74.2
420.0	+0.060	+0.395	0.935	0.230	16.7	80.1
480.0	+0.120	+0.455	0.995	0.170	17.4	85.3
540.0	+0.165	+0.500	1.040	0.125	17.9	89.2
720.0	+0.220	+0.555	1.095	0.070	16.9	94.0
1470.0	+0.240	+0.575	1.115	0.050	—	95.7

Table XXVI gives a comparison between the calculated and the experimentally found values of k , degree of hydrolysis and α . The correspondence is here somewhat better than in Tables XXII-XXIV; this may be taken as a confirmation of the assumption that it is the value of e in the former experiments which is incorrect.

Table XXVI.

$$c_d = 0.0196, k_d = 16.5 \cdot 10^{-4}, c_l = 0.0202, k_l = 15.3 \cdot 10^{-4}, e = 0.1896.$$

Time min.	$c_d - x_d$	x_d	$c_l - x_l$	x_l	$x_{\text{tot.}}$	$c - x_{\text{tot.}}$	$k \cdot 10^4$		%		α	
							calc.	f.	calc.	f.	calc.	f.
0	0.0196	—	0.0202	—	—	0.0398	—	—	—	—	-0.540	-0.540
10	0.0189	0.0007	0.0195	0.0007	0.0014	0.0384	15.6	23.0	3.5	5.2	-0.500	-0.480
30	0.0175	0.0021	0.0182	0.0020	0.0041	0.0357	15.7	17.8	10.3	11.6	-0.420	-0.405
60	0.0156	0.0040	0.0164	0.0038	0.0078	0.0320	15.7	16.3	19.5	20.2	-0.315	-0.305
90	0.0139	0.0057	0.0147	0.0055	0.0112	0.0286	15.9	16.9	28.1	29.6	-0.215	-0.195
120	0.0124	0.0072	0.0132	0.0070	0.0142	0.0256	16.0	16.4	35.7	36.5	-0.125	-0.115
150	0.0111	0.0085	0.0119	0.0083	0.0168	0.0230	15.9	16.2	42.2	42.9	-0.050	-0.040
180	0.0099	0.0097	0.0107	0.0095	0.0192	0.0206	15.9	16.2	48.2	48.9	+0.020	+0.030
210	0.0088	0.0108	0.0096	0.0106	0.0214	0.0184	16.0	16.3	53.8	54.5	+0.065	+0.095
240	0.0079	0.0117	0.0087	0.0115	0.0232	0.0166	15.8	16.0	58.3	58.8	+0.140	+0.145
270	0.0070	0.0126	0.0078	0.0124	0.0250	0.0148	15.9	16.0	62.8	63.1	+0.190	+0.195
300	0.0063	0.0133	0.0070	0.0132	0.0265	0.0133	15.9	16.2	66.6	67.4	+0.235	+0.245
360	0.0050	0.0146	0.0057	0.0145	0.0291	0.0107	15.8	16.4	73.1	74.2	+0.310	+0.325
420	0.0040	0.0156	0.0046	0.0156	0.0312	0.0086	15.8	16.7	78.4	80.1	+0.375	+0.395
480	0.0032	0.0164	0.0037	0.0165	0.0329	0.0069	15.9	17.4	82.7	85.3	+0.425	+0.455
540	0.0025	0.0171	0.0030	0.0172	0.0343	0.0055	15.9	17.9	86.2	89.2	+0.465	+0.500
720	0.0013	0.0183	0.0016	0.0186	0.0369	0.0029	15.8	16.9	92.7	94.0	+0.540	+0.555

The figures which give a comparison of the rates of hydrolysis of the different glucosides are the values of k/e (sal. f.), taken for a constant glucoside concentration. In Table XXVII these figures are given for the glucosides here examined together with values for methylglucoside, calculated by extrapolation from the

Table XXVII.

Glucoside	Conc.	k/e (sal. f.)	$v_{rel.}$
Methyl	0.04 <i>M</i>	0.0206	1.0
1-Methylpentyl	"	1.29	63.0
3-Methylpentyl	"	0.186	9.0
Methyl	0.02 <i>M</i>	0.0211	1.0
<i>d</i> -1-Methylpentyl	"	1.89	89.0
<i>l</i> -1-Methylpentyl	"	0.548	26.0
<i>d</i> -3-Methylpentyl	"	0.193	9.1
<i>l</i> -3-Methylpentyl	"	0.180	8.5

experiments of Josephson [1925, p. 59]. The relative velocities are also given in the table, the velocity of hydrolysis of methylglucoside being taken as unity.

It remains to be seen if there is a proportionality between the affinity constants of the different glucosides and their rates of hydrolysis.

REFERENCES.

- v. Falkenhausen and Neberg (1931). *Biochem. Z.* **242**, 481.
 Fischer and Raske (1909). *Ber. deutsch. chem. Ges.* **42**, 1465.
 Hardin (1908). *J. Chim. Phys.* **6**, 584.
 Helfferich (1930). *Z. physiol. Chem.* **189**, 273.
 — (1931). *Z. physiol. Chem.* **198**, 100.
 Josephson (1925). *Z. physiol. Chem.* **147**, 31.
 Mitchell and MacArthur (1932). *J. Chem. Soc.* 1669.
 Neberg, Jacobsohn and Wagner (1929). *Fermentforsch.* **10**, 492.
 Pickard and Kenyon (1911). *J. Chem. Soc.* **99**, 49.
 Veibel and Bach (1932). *Biochem. Z.* **252**, 401.
 Zemplén and Pascu (1929). *Ber. deutsch. chem. Ges.* **62**, 1613.