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Acid and Enzymic Hydrolysis of β-Glucosides. II

STIG VEIBEL and EDITH HJORTH

Department of Organic Chemistry, University of Technology, Copenhagen, Denmark

In a recent investigation Guggenheim and Wiseman [1] have measured the velocity of inversion of sucrose by strong acids and have found the velocity to be strictly first order, the first order velocity constant k_1 being related to the molarity of strong acid c by the formula

$$k_1/\text{min}^{-1} = 6.95 \cdot 10^{-3} \cdot c \cdot 10^{Bj \cdot c},$$

where B_j is a constant determined by the anion. For hydrochloric acid they found $B_j = 0.28$. If the acid contains neutral uni-univalent salts the expression

$$k_1/\text{min}^{-1} = 6.95 \cdot 10^{-3} \cdot c_H \cdot 10^{\Sigma Bj \cdot cj},$$

where c_H denotes the molarity of strong acids and c_j the molarity of each anion, was found valid, each B_j having the same value as in the formula for single acids. The formula is in accordance with Brønsteds principle of specific interaction of ions [2].

Guggenheim and Wiseman compared their results with those of other workers, *e.g.* an investigation by Leininger and Kilpatrick [3], and found good agreement within the experimental accuracy.

Previously Veibel and Frederiksen [4] investigated the acid and the enzymic hydrolysis of β-alkylglucosides. They found the velocity constants dependent on the concentration of the acid and, using some values for the activity of hydrogen ions in hydrochloric acid of molarity ranging from 0.1 *M* to 5 *M* given by Duboux [5], they found the velocity constants proportional to the activity of hydrogen ions in the solution examined. They, too, compared their results with the above mentioned investigation [3]. The American authors found at 0° $k_{1.0\ M\ HCl}/k_{0.5\ M} = 2.79$ as compared with 2.75 calculated from Duboux' values of activity of hydrogen ions. This agreement was by Veibel and Frederiksen taken as a corroboration of the dependency of k on the activity of hydrogen ions.

It seems, however, more likely that the dependency of k on the molarity of the acid is governed by the principle of specific interaction of ions than by the activity of hydrogen ions in the solution. We therefore recalculated the results of Veibel and Frederiksen, making use of the formula proposed by Guggenheim and Wiseman. From experiments with 1.0 M and 0.5 M HCl we found at 25° $B_{j\mathrm{Cl}} = 0.26_2$, not very different from the value 0.28 given by Guggenheim and Wiseman at 24.7°. From Leininger and Kilpatricks experiments is found at 25° $B_{j\mathrm{Cl}} = 0.20_2$.

Most of the experiments of Veibel and Frederiksen are made at temperatures ranging from 60° to 90°, the total range of temperature investigated being from 0° to 90°. Leininger and Kilpatrick found B_j to be dependent on the temperature. They indicate $B_j = 0.2230$ at 10°, 0.1806 at 40°. We found it possible to express the dependency on the temperature by the formula $B_j = B_{jo} + \beta \cdot T$, T being the absolute temperature. For chlorine ion we find $B_{jo\mathrm{Cl}^-} = 0.710$ and $\beta = -0.0015$. From the figures given by Leininger nad Kilpatrick we calculate $B_{jo\mathrm{Cl}^-} = 0.619$ and $\beta = -0.0014$.

In a single experiment Veibel and Frederiksen examined the influence of the addition of an uni-univalent salt on the velocity constant. They compared the hydrolysis of dimethyl-ethyl-carbinol-β-D-glucoside at 40° with 0.5 N HCl and with a solution being at the same time 0.5 N in HCl and KCl. From the formula (1) given beneath we calculate $\log(k_{0.5N\ \mathrm{HCl} + 0.5N\ \mathrm{KCl}}/k_{0.5N\ \mathrm{HCl}}) = 0.12$ or $k_{0.5N\ \mathrm{HCl} + 0.5N\ \mathrm{KCl}}/k_{0.5N\ \mathrm{HCl}} = 1.32$. Experimentally was found $k_{0.5N\ \mathrm{HCl} + 0.5N\mathrm{KCl}} = 51.9 \cdot 10^{-4}$ and $k_{0.5N\ \mathrm{HCl}} = 39.6 \cdot 10^{-4}$. The relation between these two constants is 1.31 in excellent agreement with the calculated value 1.32.

Guggenheim and Wiseman found k_1 for 0.1 N HCl to be linear in the concentration of sucrose, the constants increasing some 5 % with an increase in sucrose concentration from 0.1 M to 0.2 M. Veibel and Frederiksen, on the other hand, found no significant dependency of k on the concentration of glucoside, and if a dependency could be found it was a slight decrease in k with an increase of glucoside concentration from 0.1 M to 0.2 M.

In table 1 we summarise all the experiments of Veibel and Frederiksen, calculated according to the formula

$$k_1 \cdot 10^4/\mathrm{min}^{-1} = a \cdot c_{\mathrm{HCl}} \cdot 10^{(0.710 - 0.0015T)c\mathrm{HCl}} \qquad (1)$$

In these experiments only alkyl-β-D-glucosides were examined. We have extended the investigation to phenol- and *o*-cresol-β-D-glucoside, and Table 1 summarises also the results of the new experiments.

The experimentally found velocity constants used in the calculations are underlined. For temperatures different from the temperature of the underlined experiment the k_1-values are calculated from the expression log (k_{T_1}/k_{T_2})

Table 1. Velocity constants for the acid hydrolysis of β-glucosides,

$$log\ k/min^{-1} = log\ a + log\ c_{HCl} + B_j \cdot c_{HCl}.$$
$$B_j = B_{jo} + \beta \cdot T;\ B_{jo} = 0.710,\ \beta = -0.0015.$$

Glucoside	log a	Q cal	60° $k \cdot 10^4$		70° $k \cdot 10^4$		80° $k \cdot 10^4$		90° $k \cdot 10^4$	
Methyl			exp	calc	exp	calc	exp	calc	exp	calc
1.0 *M* HCl	0.9852−4	32520	0.91	0.97	3.93	3.92	14.6	—	53.5	50.6
0.5 *M* HCl				0.38	1.51	1.57	6.01	5.95		
Propyl										
1.0 *M* HCl	0.1275−3	32520	1.20	1.35	5.27	5.45	20.3	—	69.0	70.3
0.5 *M* HCl					1.86	2.18	7.8	8.3		
Isopropyl										
1.0 *M* HCl	0.3502−3	31800	2.06	2.39	8.42	9.37	33.9	—	113.5	114.0
0.5 *M* HCl					3.09	3.74	13.1	13.8		
Diethyl-carbinol			50°		60°		70°		80°	
1.0 *M* HCl	0.5022−3	31800	0.74	0.80	3.08	3.40	13.4	13.3	48.1	—
0.5 *M* HCl							5.05	5.31	19.0	19.5
Trimethyl-carbinol			20°		30°		40°		50°	
1.0 *M* HCl	0.1750−3	30340	0.85	1.00	4.97	5.37	26.0	—	116.3	113.7
0.5 *M* HCl							9.5	9.9	42.8	44.0
Dimethyl-ethyl-carbinol			0°		20°		30°		40°	
1.0 *M* HCl	0.8081−3	30340	0.098	0.101	4.25	4.29	23.0	23.1	111.7	—
0.5 *M* HCl							8.1	8.6	39.6	42.4
Neopentyl			60°		70°		80°		90°	
1.0 *M* HCl	0.2333−3	32520	1.74	1.72	6.44	6.95	25.9	—	89.5	89.6
1.0 *M* HCl		30340		2.07		7.61		—		82.3
Phenol										
1.0 *M* HCl	0.8099−3	30340	7.51	7.81	27.2	28.7	97.7	—		
1.0 *M* HCl		32520		6.48		26.2		—		
0.5 *M* HCl		30340		3.07	12.4	11.5	40.7	39.7		
0.5 *M* HCl		32520				10.5		39.7		
			40°		60°		80°			
o-Cresol	0.7625−3									
1.0 *M* HCl		30340	0.36	0.40	6.59	7.00	87.6	—		
1.0 *M* HCl		32520		0.26		5.81		—		
0.5 *M* HCl		30340	0.13	0.15	2.63	2.75	36.7	35.6		
0.5 *M* HCl		32520		0.10		2.28		35.6		

$= Q(T_1-T_2)/2.303 \cdot R \cdot T_1 \cdot T_2$, the activation energy Q being calculated from two experiments with as great temperature difference as possible, *viz.* 40°. It is obvious from the table that it is not permissible to use the same value of Q over the whole range of temperature examined. Leininger and Kilpatrick [3] have for the inversion of sucrose determined the variation of Q with the temperature. They found Q decreasing with increasing temperature, $\Delta Q/\Delta T$ being approximately -70 cal pr. degree at $T = 283°$, decreasing with increasing temperature. Moelwyn-Hughes [6] found the larger value -94 cal pr. degree at $T = 307°$. The accuracy of the determinations of Veibel and Frederiksen not being sufficient to allow the calculation of the temperature dependency on Q we have used the average value for the temperature range examined, but the discrepancy between calculated and found values is considerably greater at the lower end of the temperature range than at the upper end, in agreement with the statement that the activation energy increases with decreasing temperature.

Leininger and Kilpatrick found the activation energy dependent on the concentration of hydrochloric acid, Q decreasing some 500 cal pr. unity of molarity in the range 0 M to 3 M hydrochloric acid. This means for activation energies about 30 000 cal that k will decrease some 30 % when 0.5 M HCl is used in stead of 1.0 M HCl. From Table 1 is seen that we too find an influence of the concentration of acid on k, but not so great as that stated by Leininger and Kilpatrick.

Table 2. Acid hydrolysis of some β-D-glucosides. HCl 1.0 N *if not otherwise stated.*

	$k \cdot 10^4/\text{min}^{-1}$				Q
	61.2°	60°	77°	80°	
Methyl-β-D-glucoside					
Kuhn and Sobotka [7]			4.6	(6.8)	
0.5 *N* HCl					
Moelwyn-Hughes [8]		1.0		18.2	33 370
Phenol-β-D-glucoside					
Kuhn and Sobotka [7]					
0.5 *N* HCl			24.3	(35.0)	
Scheiber [9]	9.4	(8.0)			
o-Cresol-β-D-glucoside					
Scheiber [9]	7.8	(6.7)			

(The values in brackets are calculated assuming the Q-values found in this investigation.)

The acid hydrolysis of some β-D-glucosides has been investigated previously by Kuhn and Sobotka [7], by Moelwyn-Hughes [8], and by Scheiber [9]. Table 2 summarises the results.

By comparison with Table 1 it is seen that the values found by Veibel and Frederiksen for methyl-glucoside and the values found by us for phenol- and *o*-cresol-glucoside agree tolerably well with the values found by other investigators. The accuracy obtained by Scheiber cannot be regarded as sufficient as there is no guarantee for the temperature being kept really constant (the only indication is: boiling chloroform) and, moreover, only two observations are used for the calculation of k.

More recently Heidt and Purves [10] have investigated the acid hydrolysis of some glycosides, among which were methyl-β-D-glucoside and phenol-β-D-glucoside. Using roughly 0.1 N HCl and temperatures from 55° to 96° the American authors found the velocity constant proportional to the concentration of hydrochloric acid, and calculated values for half life time at 60° in 0.05 N HCl were indicated. Besides, they indicate the activation energy and the constant B in the Arrhenius-equation. Table 3 gives their figures and the value of $k \cdot 10^4$ calculated from the half life time indicated. Using formula (1) we have from these data calculated $k \cdot 10^4$ in 0.5 N HCl at 60°.

Table 3. Heidt and Purves' values for acid hydrolysis of glucosides.

	Half life time 0.05 N HCl 60° min	$k \cdot 10^4$ 0.05 N HCl 60°	Q	B	$k \cdot 10^4$ 0.5 N HCl 60°
Methyl-β-D-glucoside	104 000	0.029	33 460	18.07	0.36
Phenol-β-D-glucoside	11 500	0.26	32 200	18.20	3.2

Both for methyl-glucoside and for phenol-glucoside the values calculated for 0.5 N HCl agree pretty well with the values found by us.

Veibel and Frederiksen have for the constant B in the Arrhenius equation calculated values approximately 16.0 for glucosides of primary and secondary alcohols and 17.5 for glucosides of tertiary alcohols. From Table 1 is seen that the corresponding Q-values are 32 520, 31 800, and 30 340 cal. respectively.

In Table 4 we have calculated the B-values for phenol- and for *o*-cresol-β-D-glucoside from the formula $\log k = \frac{-Q}{2.303\ RT} + B$, using for k values with the second as unit of time. It is seen that the average value of B is 15.2

for the arylglucosides, *i.e.* considerably lower than the values for alkylglucosides.

From the formula the influence of variation of Q and of B on k may be calculated. When B is kept constant a decrease in activation energy from 32 520 cal to 30 340 cal means an increase in k by a factor 25, and when Q is kept constant a decrease of B from 17.5 to 15 means a decrease of k by a factor 315.

Table 4. Values of k, Q, *and* B *for arylglucosides at different temperatures.* k *is calculated with the second as unit of time and with natural logarithms.*

Aglucon	1.0 *N* HCl				0.5 *N* HCl			Enzymic			
	t	$k \cdot 10^5$	*Q*	*B*	$k \cdot 10^5$	*Q*	*B*	*t*	$k_3 \cdot 10^3$	*Q*	*B*
Phenol 0.1 *M*	60°	2.88	29600	14.89	—	—	—	30°	2.44	11980	6.03
	70°	10.4	29600	14.88	4.76	29600	14.54	20°	1.24		
	80°	37.4	29600	14.90	15.6	29600	14.52				
				14.9			14.5				6.0
Phenol 0.05 *M*	40°	0.14	29600	14.82	—	—	—				
	60°	3.02	29600	14.91	1.34	29600	14.56				
	70°	10.8	29600	14.89	4.61	29600	14.52				
	80°	36.7	29600	14.89	15.4	29600	14.52				
				14.9			14.5				
o-Cresol 0.1 *M*	40°	0.13	30520	15.44	0.05	30520	15.02	30°	13.4	10790	5.92
	60°	2.53	30520	15.43	1.01	30520	15.03	20°	7.3		
	80°	33.6	30520	15.43	14.1	30520	15.05				
				15.4			15.0				5.9
o-Cresol 0.05 *M*	40°	0.13	30520	15.44	0.05	30520	15.03				
	60°	2.52	30520	15.43	1.01	30520	15.03				
	80°	33.9	30520	15.43	13.7	30520	15.04				
				15.4			15.0				

A comparison of Table 1 and Table 4 shows that the rapid hydrolysis of glucosides of tertiary alcohols is due to the combined effect of a relatively small activation energy and a great constant of action. For the arylglucosides examined Q has the same value as for glucosides of tertiary alcohols but the B-value is considerably lower than the B-value of glucosides of tertiary alcohols and even of primary and secondary alcohols. In Table 1 we have tentatively calculated the velocity constants for arylglucosides at different temperatures, assuming for Q first the value found for glucosides of tertiary alcohols and next the value found for glucosides of primary alcohols. It is seen that the agreement between calculated and found values is considerably better when

the value for glucosides of tertiary alcohols is used than with the value for glucosides of primary alcohols.

For neopentylglucoside the velocity of enzymic hydrolysis is of the same order of magnitude as for glucosides of tertiary alcohols, the velocity of acid hydrolysis of the same order of magnitude as for glucosides of primary and secondary alcohols. The B-value is for acid hydrolysis somewhat lower than the B-values for glucosides of primary and secondary alcohols. In Table 1 we have calculated the velocity constants at different temperatures, assuming first the Q-value of glucosides of primary alcohols and next the Q-value of glucosides of tertiary alcohols. It is seen that here the best agreement is obtained by assuming the Q-value of glucosides of primary alcohols whereas Veibel and Frederiksen for the enzymic hydrolysis of neopentylglucoside found a Q-value approximately the same as for glucosides of tertiary alcohols.

As mentioned above the agreement between the velocity constants found by Heidt and Purves and by us is tolerably good. Considering the Q- and B-values there is, however, disagreement, most pronounced for phenol-β-D-glucoside. The American authors have found for phenolglucoside a Q-value slightly lower than the Q-value for methylglucoside, the B-values being practically identical. We, on the other hand, find the Q-value for phenolglucoside considerably lower than the Q-value for methylglucoside and the B-value somewhat lower than the value of B for glucosides of primary alcohols. But the B-values found by Heidt and Purves are 18.07 and 18.20 whereas we find the values 16.0 and 15.2. From this difference in B we should expect the velocity constants found by Heidt and Purves to be 300—400 times higher than the constants found by us. We are not able to give an explanation to this discrepancy, the experimental evidence given by Heidt and Purves not allowing a recalculation of their Q- and B-values. The tolerance given by them for their values seems to exclude experimental error as an explanation.

From the velocity constants for the enzymic hydrolysis of phenol- and o-cresol-β-D-glucoside at 30° and at 20° we calculated the heat of activation and, with the same reservation as in the above mentioned investigation of the enzymic hydrolysis of alkylglucosides [4], the B-values in the Arrhenius-equation. Besides,we have determined the affinity constants K_m and K_{m2} for the compounds enzyme-phenol-β-D-glucoside and enzyme-phenol respectively so that the k_3-value for the hydrolysis of phenol-β-D-glucoside may be calculated and compared with the k_3-value for the hydrolysis of o-cresol-β-D-glucoside previously determined. The results are summarised in Table 5.

It is seen from Table 4 that by acid hydrolysis phenol-β-D-glucoside is hydrolysed somewhat faster than o-cresol-β-D-glucoside. By enzymic hydrolysis catalysed by almond emulsin o-cresol-β-D-glucoside is hydrolysed considerably

Table 5. Enzymic hydrolysis of aryl-β-D-glucosides. Sweet almond emulsin. Glucoside 0.0400 M. Sal.f. = 0.60. Phosphate-citrate buffer, pH 4.4.

		Phenolglucoside	o-Cresolglucoside
K_m 30°		0.10	0.013
K_{m_2} 30°		0.10	0.020
$10^2 \cdot k_{obs}/e$ (Sal.f.)	30°	43.6	756
	20°	22.6	414
$10^2 \cdot k_3$	30°	6.37	34.8
	20°	3.24	18.9
$k_{obs\ cresol}/k_{obs\ phenol}$	30°	17.3	
	20°	18.4	
$k_{3\ cresol}/k_{3\ phenol}$	30°	5.4	
	20°	5.8	

faster than phenol-β-D-glucoside, the k_3-value for o-cresol-β-D-glucoside being 5.5 times greater than the corresponding value for phenol-β-D-glucoside, whereas the factor for the k_{obs}-values is 18. The effect of the methyl group in o-position to the glucosidic linkage is consequently partly due to an increase in the affinity between enzyme and substrate and partly to an increase in the velocity of dissociation of the enzyme-substrate compound into enzyme, glucose, and aglucon.

Table 6. Acid hydrolysis.

Phenolglucoside 0.05 *M*. Hydrochloric acid 1.0 *N*. 80°

t min	*a*	*x*	*c*−*x*	$k \cdot 10^4$ *	*x'*	*v*	$k \cdot v$
0	−1.40	—	2.09	—	—	—	—
10	−0.99	0.41	1.68	94.9	0.196	0.0607	5.77
20	−0.66	0.74	1.35	95.0	0.354	0.0793	7.53
30	−0.38	1.02	1.07	96.9	0.488	0.0760	7.37
50	−0.02	1.38	0.71	93.8	0.660	0.0542	5.08
70	0.24	1.64	0.45	95.3	0.785	0.0308	2.94
100	0.47	1.87	0.22	97.8	0.895	0.0108	1.05
120	0.59	1.99	0.10	110.0	0.952	0.0030	0.33

mean value 97.7 $\qquad \frac{\Sigma\, k \cdot v}{\Sigma\, v} = 95.5$

* In this and the following tables k' means constants calculated from zero time to time of sample-taking, whereas k means constants calculated from point to point. x' is degree of hydrolysis.

EXPERIMENTAL

Substrate. Phenol-β-D-glucoside and *o*-cresol-β-D-glucoside were prepared by current methods and showed the physical constants reported in the litterature.

Enzyme. The β-glucosidase used in this investigation was prepared from sweet almonds by Dr. Shih-Lin Yang, see the following paper. The preparation had a Sal.f. 0.60, corresponding to a β-glucosidase value 2.00.

Methods. For the acid hydrolysis the technique indicated by Veibel and Frederiksen [4] was employed, for the enzymic hydrolysis that indicated by Veibel and Lillelund [11,12]. The following tables are examples from the laboratory journal. In experiments with acid hydrolysis the constants are calculated as indicated by Christiansen [13] who stresses the importance of giving more weight to the range 20—60 % reaction than to the 0—20 % and 60—100 % ranges, due to the greater reading accuracy obtainable in the preferred range.

Table 7. Acid hydrolysis.

o-Cresolglucoside 0.10 *M*. Hydrochloric acid 0.5 *N*. 40°.							
t min	*a*	*x*	*c*−*x*	$k \cdot 10^4$	*x'*	*v*	$k \cdot v$
0	−2.68	—	4.02	—	—	—	—
1440	−2.52	0.16	3.86	0.12_2	0.040	0.0162	0.00198
2880	−2.36	0.32	3.70	0.12_5	0.080	0.0305	0.00381
4320	−2.18	0.50	3.52	0.13_4	0.124	0.0443	0.00591
5760	−2.03	0.65	3.37	0.13_3	0.162	0.0563	0.00749
7200	−1.87	0.81	3.21	0.13_6	0.202	0.0622	0.00844
8640	−1.72	0.96	3.06	0.13_7	0.239	0.0686	0.00942
10080	−1.60	1.08	2.94	0.13_5	0.269	0.0726	0.00979

mean value 0.13_2 $\qquad \frac{\Sigma\, k \cdot v}{\Sigma\, v} = 0.13_4$

From the values given in Table 8 k_3 is calculated, using the expression

$$k_3 = k_{obs}\,(K_m + c + (K_m/K_{m1} + K_m/K_{m2} - 1)c_x/e(\text{Sal.f.}),$$

k_{obs} being the constant calculated from point to point, c_x the mean concentration of the products of hydrolysis in the points used for the calculation of k_{obs}, and K_m, K_{m1}, K_{m2} the dissociation constants of the compounds enzyme-*o*-cresolglucoside, enzyme-glucose, and enzyme-*o*-cresol respectively. These values are at pH 4.4 0.013, 0.20, and 0.020. The k_3-values found are at 30° $34.8 \cdot 10^{-2}$, at 20° $18.9 \cdot 10^{-2}$, $k_{3_{30}}/k_{3_{20}} = 1.84$. $k_{3_{30}}$ was previously (Veibel and Lillelund [7]) found to be $37.5 \cdot 10^{-2}$.

Table 8. Enzymic hydrolysis. 30° and 20°. o-Cresolglucoside 0.0394 M. *Phosphate-citrate buffer pH 4.4. Emulsin 0.0056 g in 50 ml (20°) or 0.0028 g in 50 ml (30°). Sal.f. 0.60.*

$a_{beg} = -1.305°$, $a_{end} = +0.610°$, $a_{emulsin} < 0.005°$.

	30°				20°			
t min	a	x	$c-x$	$k' \cdot 10^3$	a	x	$c-x$	$k' \cdot 10^3$
0	−1.305	—	1.915	—	−1.305	—	1.915	—
10	−0.800	0.505	1.410	13.3	−0.760	0.545	1.370	14.5
20	−0.445	0.860	1.045	13.2	−0.395	0.910	1.005	14.0
30	−0.180	1.125	0.790	12.9	−0.110	1.195	0.720	14.2
40	0.000	1.305	0.610	12.4	0.095	1.400	0.515	14.3
60	0.285	1.590	0.325	12.8	0.315	1.620	0.295	13.5
90	0.460	1.765	0.150	12.3	0.490	1.795	0.120	13.3
120	0.545	1.850	0.065	12.2	0.565	1.870	0.045	13.6
		mean value		12.7		mean value		13.9
		k/e (Sal.f.)		$= 756 \cdot 10^{-2}$		k/e (Sal.f.)		$= 414 \cdot 10^{-2}$

$k_{30}/k_{20} = 1.83$

The corresponding values for phenol-β-D-glucoside are $k'_{obs_{30}}/e$(Sal.f.) $= 43.6 \cdot 10^{-2}$, $k'_{obs_{20}}/e$(Sal.f.) $= 22.6 \cdot 10^{-2}$, $k'_{obs_{30}}/k'_{obs_{20}} = 1.93$, $k_{3_{30}}/e$(Sal.f.) $= 6.37 \cdot 10^{-2}$, $k_{3_{20}}/e$(Sal.f.) $= 3.24 \cdot 10^{-2}$, $k_{3_{30}}/k_{3_{20}} = 1.97$. For the determination of the affinity constant for β-glucosidase-phenol-β-D-glucoside the velocity constants of hydrolysis for solutions of the glucoside, 0.0100, 0.0200, 0.0300, 0.0400, 0.0600 and 0.0800 M, were determined. Table 9 and Fig. 1 give the result.

Table 9. Determination of K_m.

Phenol-β-D-glucoside 30°. Phosphate-citrate buffer pH 4.4. $e = 0.0119$. Sal.f. $= 0.60$.

$c_{glucoside}$	$k' \cdot 10^4$	$\frac{10^2 \cdot k}{e \text{ (Sal.f.)}}$	e(Sal.f.)$/k'$	$k'(k_m + c) \times 10^2$	$10^2 \cdot k_3$
0.0100	39.8	55.7	1.79	6.13	5.90
0.0200	35.4	49.6	2.02	5.95	5.88
0.0300	31.8	44.5	2.25	5.79	5.83
0.0400	29.9	41.9	2.39	5.86	5.95
0.0600	27.4	38.4	2.61	6.14	5.99
0.0800	24.0	33.6	2.98	6.05	5.94
			mean value	5.99	5.92

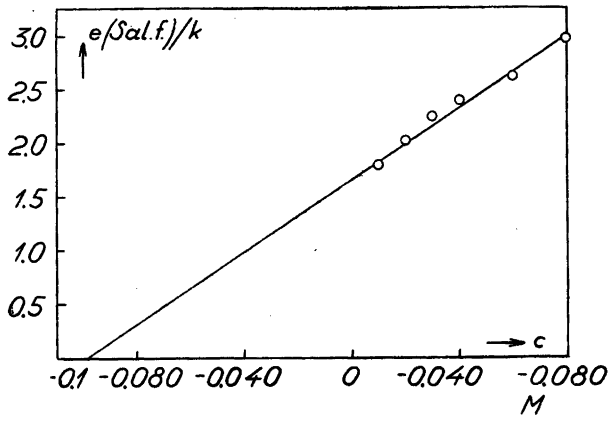

Fig. 1. Determination of K_m. *Phenol-β-D-glucoside.*

From Fig. 1 K_m is determined to 0.10. In the 5. column in Table 9 k_3 is calculated from the simplified expression $k_3 = k'(K_m + c)$, in column 6 the values of k_3 are calculated from the more accurate expression indicated above. The experiment recorded in Table 10 gives for K_{m2} the value 0.10, and for K_{m1} the value 0.20 found previously [12] is used. It is seen that the difference between the two series of k_3-values does not exceed 4 %.

Finally, we summarise in Table 11 all results obtained with enzymic hydrolysis of phenol-β-D-glucoside.

Table 10. Determination of K_{m_2}.

Phenol-β-D-glucoside 30°. Phosphate-citrate buffer pH 4.4. e = 0.0069, Sal.f. = 0.60.

c_{Phenol}	$k \cdot 10^4$	$k/k_{ph}-1$	$\frac{k/k_{ph}-1}{c_{ph}}$
0.00	16.1	—	—
0.01	14.7	0.095	9.5
0.02	13.7	0.175	8.8
0.04	12.8	0.255	6.4
0.06	11.7	0.375	6.3
0.08	10.6	0.520	6.5

mean value 7.5

$$K_{m_2} = \frac{K_m \cdot c_{ph}}{(K_m + c)\ (k/k_{ph}-1)}$$

$$= \frac{0.10}{0.14 \cdot 7.5} = 0.10$$

Table 11. Summary of results obtained with enzymic hydrolysis of phenol-β-D-glucoside.

	$c_{glucoside}$	c_{phenol}	$10^2 \cdot k'$ e/(Sal.f.)	$10^2 \cdot k$ e/(Sal.f.)	$10^2 \cdot k_3/e$ (Sal.f.)
30°	0.0400	0.00	43.6	43.6	6.39
	0.0100	0.00	55.7	53.1	5.90
	0.0200	0.00	49.6	48.6	5.88
	0.0300	0.00	44.5	43.6	5.83
	0.0400	0.00	41.9	41.7	5.95
	0.0600	0.00	38.4	36.7	5.99
	0.0800	0.00	33.6	32.4	5.94
	0.0400	0.00	38.9	38.6	5.48
	0.0400	0.01	35.5	34.8	5.27
	0.0400	0.02	33.1	32.4	5.22
	0.0400	0.04	30.9	30.0	5.44
	0.0400	0.06	28.3	28.5	5.73
	0.0400	0.08	25.6	26.1	5.77
	mean value (for k and k' only underlined experiments)		41.5	41.3	5.75
20°	0.0400	0.00	22.6	22.1	3.24

SUMMARY

The acid hydrolysis of phenol- and *o*-cresol-β-D-glucoside has been investigated. The former glucoside is hydrolysed by acids somewhat faster than the latter, whereas when hydrolysed with β-glucosidase from sweet almonds as catalysator *o*-cresol-glucoside is hydrolysed some 18 times as fast as phenol-glucoside.

The velocity constants of acid hydrolysis are of the same order of magnitude as for glucosides of primary and secondary alcohols but inferior to the velocity constants for glucosides of tertiary alcohols by 2—3 orders of magnitude.

The heat of activation for the acid hydrolysis of phenol- and *o*-cresol-β-D-glucoside is closer to the heat of activation for the hydrolysis of glucosides of tertiary alcohols than to the heat of activation for the hydrolysis of glucosides of primary and secondary alcohols.

The constants of action for the hydrolysis of the aryl-β-D-glucosides examined are lower than the constants of action for the hydrolysis of glucosides of not only tertiary but also primary and secondary alcohols. This is in disagreement with the findings of Heidt and Purves, who for phenol- and methyl-β-D-glucoside indicate constants of action identical within the limit of error.

The heat of activation and the "constants of action" for the enzymic hydrolysis of the two aryl-β-D-glucosides are closer to the corresponding values for the enzymic hydrolysis of glucosides of primary and secondary alcohols than to those of tertiary alcohols. The difference in velocity of enzymic hydrolysis of *o*-cresol- and phenol-β-D-glucoside is due partly to the greater affinity of the β-glucosidase to *o*-cresol-β-D-glucoside than to phenol-β-D-glucoside, partly to a greater k_3-value for the former glucoside than for the latter.

REFERENCES

1. Guggenheim, E. A., and Wiseman, L. A. *Proc. Roy. Soc.* **A 203** (1950) 17.
2. Brønsted, J. N. *J. Am. Chem. Soc.* **44** (1922) 877.
3. Leininger, P. M., and Kilpatrick, M. *J. Am. Chem. Soc.* **60** (1938) 2891.
4. Veibel, S., and Frederiksen, E. *Kgl. Danske Videnskab. Selskab. Mat.-fys. Medd.* **19** (1941) no. 1.
5. Duboux, M. *Bull. Soc. Vaud. Sc. Nat.* **59** (1937) 49.
6. Moelwyn-Hughes, E. A. *Ztschr. physik. Chem.* **B 26** (1934) 281.
7. Kuhn, R., and Sobotka, H. *Ztschr. physiol. Chem.* **109** (1924) 65.
8. Moelwyn-Hughes, E. A. *Transact. Farad. Soc.* **25** (1929) 503.
9. Scheiber, H. E. *Dissertation*, Leipzig (1935).
   Helferich, B., and Scheiber, H. E. *Ztschr. physiol. Chem.* **226** (1934) 272.
10. Heidt, L. J., and Purves, C. B. *J. Am. Chem. Soc.* **66** (1944) 1385.
11. Veibel, S., and Lillelund, H. *Kgl. Danske Videnskab. Selskab. Mat.-fys. Medd.* **17** (1940) no. 6.
12. Veibel, S., and Lillelund, H. *Enzymologia* **9** (1940) 161.
13. Christiansen, J. A. *Ztschr. physik. Chem.* **A 189** (1941) 126.

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