221. *Exponential Analysis applied to the Asymmetric Hydrolysis of Some β-Glucosides with Emulsin.*

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THE rates of hydrolysis of the *d*-glucosides of *d*- and *l*-methyl-*n*-hexylcarbinol with emulsin have already been examined by Mitchell (*J. Biol. Chem.*, 1929, **82**, 727), and it was thought that an extension of the work to the glucosides of other optically active aliphatic alcohols would be of interest. For this purpose it was desirable to avoid as far as possible the tedious and costly process of resolving the alcohols. Since in the previous experiments (*loc. cit.*) the unimolecular reaction constants did not deviate from the mean by more than ± 1·7%, the possibility was considered of applying exponential analysis to the hydrolysis–time curve for the mixture of glucosides obtained from a racemic alcohol. It was decided to test the validity of the method with the previously prepared *dl*-methyl-*n*-hexylcarbinyl-*d*-β-glucoside.

A small quantity (0·0818 g.) of this glucoside was weighed into a 50-c.c. graduated flask, which was then placed in a thermostat at 37°. The enzyme solution was prepared by mixing 0·100 g. of emulsin with 100 c.c. of water, filtering it, and allowing the filtrate to come to 37° in the thermostat. The hydrolysis experiment was started by filling the graduated flask to the mark with the emulsin solution. 2 C.c. were withdrawn at intervals during about 10 hours, and the volume T_n of 0·01*N*-sodium thiosulphate solution equivalent to the amount of glucose set free was determined by the method of Maclean (*Bio-*

chem. J., 1919, **13**, 135). The final reading T_∞ was obtained some 24 hours later. Table I (A) gives the values of T_n and $T_\infty - T_n$ at the times shown. The differences $T_\infty - T_n$, which are proportional to the concentrations of unchanged glucoside, were plotted against time, and the method of exponential analysis outlined by Walsh (*Proc. Physical Soc.*, 1919, **32**, 26) was applied, it being assumed that two components are present, *i.e.*, that the curve is of the form

$$y = a_1 e^{-k_1 t} + a_2 e^{-k_2 t} \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

where a_1, a_2, k_1, k_2 are constants, t is time, and y is $T_\infty - T_n$.

Let y_0, y_1, y_2, y_3 be the values of y corresponding to the values 0, d, $2d$, $3d$ of t, zero time and d being arbitrarily chosen. Then where,

$$e^{-k_1 d} \equiv \alpha_1,\ e^{-k_2 d} \equiv \alpha_2 \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

we have

$$\left.\begin{aligned} y_0 &= a_1 + a_2 \\ y_1 &= a_1\alpha_1 + a_2\alpha_2 \\ y_2 &= a_1\alpha_1^2 + a_2\alpha_2^2 \\ y_3 &= a_1\alpha_1^3 + a_2\alpha_2^3 \end{aligned}\right\} \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

Let p, q be such that α_1, α_2 are the roots of the equation

$$f(x) \equiv x^2 + px + q = 0$$

then $y_2 + py_1 + qy_0 = a_1 f(\alpha_1) + a_2 f(\alpha_2) = 0$

and $y_3 + py_2 + qy_1 = a_1\alpha_1 f(\alpha_1) + a_2\alpha_2 f(\alpha_2) = 0$

Elimination of p and q gives

$$\begin{vmatrix} x^2 & x & 1 \\ y_2 & y_1 & y_0 \\ y_3 & y_2 & y_1 \end{vmatrix} = 0$$

The roots of this equation, α_1, α_2, are thus easily obtained, and the values so found give k_1 and k_2 from equations (2) and a_1 and a_2 from any two of equations (3).

Probability Correction.—This exponential analysis being based only on four points from the mean curve, and being rather sensitive even to slight error in the chosen points, it is desirable to use the set of 4 parameters so obtained as a first approximation to the set which gives an equation in closest agreement with all the available reliable data. The following method was adopted. Let $\phi(a_1, a_2, k_1, k_2) \equiv a_1 e^{-k_1 t} + a_2 e^{-k_2 t}$, where (1) is the equation best representing the data of hydrolysis. Further, let $\bar{a}_1$, $\bar{a}_2$, $\bar{k}_1$, $\bar{k}_2$ be the parameter set obtained by preliminary exponential analysis, and let

$$\left.\begin{aligned} a_1 &\equiv \bar{a}_1(1 + \beta_1) \qquad k_1 \equiv \bar{k}_1(1 + \lambda_1) \\ a_2 &\equiv \bar{a}_2(1 + \beta_2) \qquad k_2 \equiv \bar{k}_2(1 + \lambda_2) \end{aligned}\right\} \quad . \quad . \quad . \quad (4)$$

then, where β_1, β_2, λ_1, λ_2 are small,

$$\begin{aligned}\delta_n &\equiv \phi(a_1, a_2, k_1, k_2)_{t=t_n} - \phi(\bar{a}_1, \bar{a}_2, \bar{k}_1, k_2)_{t=t_n} \\ &= \left\{\frac{\partial\phi}{\partial\bar{a}_1}\cdot\bar{a}_1\beta_1 + \frac{\partial\phi}{\partial\bar{a}_2}\cdot\bar{a}_2\beta_2 + \frac{\partial\phi}{\partial\bar{k}_1}\cdot\bar{k}_1\lambda_1 + \frac{\partial\phi}{\partial\bar{k}_2}\cdot\bar{k}_2\lambda_2\right\}_{t=t_n} \\ &\qquad \text{to a first order of approximation} \\ &= \beta_1(\bar{a}_1e^{-\bar{k}_1t_n}) + \beta_2(\bar{a}_2e^{-\bar{k}_2t_n}) + \lambda_1(-\bar{a}_1k_1t_ne^{-\bar{k}_1t_n}) + \lambda_2(-\bar{a}_2\bar{k}_2t_ne^{-\bar{k}_2t_n}) \quad (5) \\ &\equiv l_{n1}\beta_1 + l_{n2}\beta_2 + l_{n3}\lambda_1 + l_{n4}\lambda_2\end{aligned}$$

For each point $P_n(t_n, y_n)$ of some 16 readings, an "equation of condition" of type (5) in β_1, β_2, λ_1, λ_2 was thus formed, t_n being calculated from P (the first of the 4 selected points for the preliminary exponential analysis) as zero, and δ_n being

$$y_n - (\bar{a}_1e^{-\bar{k}_1t_n} + \bar{a}_2e^{-\bar{k}_2t_n})$$

From the 16 equations of condition were formed, as usual in the method of least squares (Whittaker and Robinson, "The Calculus of Observations," 1924, p. 209), the 4 "normal equations"

$$u_{r1}\,.\,\beta_1 + u_{r2}\cdot\beta_2 + u_{r3}\,.\,\lambda_1 + u_{r4}\,.\,\lambda_2 = v_r \quad . \quad . \quad (6)$$
$$(r = 1, 2, 3, 4)$$

where $$u_{rs} \equiv \sum_{n=1}^{16} l_{nr}\,.\,l_{ns} \text{ and } v_r \equiv \sum_{n=1}^{16} l_{nr}\,.\,\delta_n$$

Equations (6) were then solved for β_1, β_2, λ_1, λ_2 either as ratios of determinants or by successive approximation, and the results inserted in (4) gave the required best set of parameters a_1, a_2, k_1, k_2, whence equation (1).

For the approximate exponential analysis of the octylglucoside graph, the following set of 4 points was selected, the time for the first point P being taken as zero. P is chosen some distance from the beginning so as to avoid initial irregularities.

t (hours)	0	2·5	5	7·5
y	4·795	1·845	1·078	0·640

The values obtained were

$$\bar{a}_1 = 3{\cdot}054 \qquad \bar{k}_1 = 0{\cdot}208$$
$$\bar{a}_2 = 1{\cdot}741 \qquad \bar{k}_2 = 1{\cdot}610$$

After application of the probability correction, the equation for the curve became

$$y = 3{\cdot}13e^{-0{\cdot}213t} + 1{\cdot}65e^{-1{\cdot}84t}$$

from which $k_2/k_1 = 8{\cdot}6$. Table II (A) shows the extent of agreement between the mean curve for the octylglucoside and the values calculated from the final equation.

From these results, it would appear that the original assumption that two exponential components are present is justified. The two β-glucosides, *d*- and *l*-methyl-*n*-hexylcarbinyl-*d*-glucosides, in the mixture are hydrolysed at different rates, and the ratio of the reaction constants (8·6) obtained by exponential analysis is in good agreement with the value (8·4) previously obtained when the two glucosides were hydrolysed separately.

EXPERIMENTAL.

dl-*Methyl*-n-*butylcarbinyl*-d-β-*glucoside*.—The alcohol was obtained by the reduction of methyl *n*-butyl ketone (Bentley, J., 1895, **67**, 265). *Tetra-acetyl*-β-*methyl*-n-*butylcarbinyl*-d-*glucoside* was then prepared exactly as described previously for the methyl-*n*-hexylcarbinyl compound; m. p. 99° (Found : C, 55·8; H, 7·3. $C_{20}H_{32}O_{10}$ requires C, 55·6; H, 7·4%). Subsequent removal of the acetyl groups gave β-*methyl*-n-*butylcarbinyl*-d-*glucoside*, m. p. 115° (Found : C, 54·6; H, 9·0. $C_{12}H_{24}O_6$ requires C, 54·6; H, 9·1%).

For the hydrolysis experiment, 0·0701 g. of this glucoside was weighed into a 50-c.c. graduated flask, and a solution of emulsin (0·0504 g. in 100 c.c.) was added. The readings given in Table I (B) were then taken as described for the octylglucoside. For the preliminary exponential analysis, the following 4 points were selected

t (hours)	0	2	4	6
y	5·465	2·150	0·895	0·388

and from these

$$\bar{a}_1 = 3{\cdot}517 \qquad \bar{k}_1 = 0{\cdot}384$$
$$\bar{a}_2 = 1{\cdot}948 \qquad \bar{k}_2 = 0{\cdot}662$$

After application of the probability correction, the following equation was obtained

$$y = 3{\cdot}49e^{-0{\cdot}398t} + 1{\cdot}97e^{-0{\cdot}602t}$$

from which $k_2/k_1 = 1{\cdot}5$. The agreement between the mean curve and the calculated values is shown in Table II (B).

dl-*Methylethylcarbinyl*-d-β-*glucoside*.—In this case the alcohol was obtained by the reduction of methyl ethyl ketone. The *tetra-acetyl* compound was then prepared, m. p. 125° (Found : C, 53·7; H, 7·0. $C_{18}H_{28}O_{10}$ requires C, 53·5; H, 6·9%). Removal of the acetyl groups gave the *glucoside*, m. p. 95° (Found : C, 50·6; H, 8·5. $C_{10}H_{20}O_6$ requires C, 50·8; H, 8·5%).

For the hydrolysis experiment, 0·1311 g. of this glucoside was weighed into a 50-c.c. flask, and a solution of emulsin (0·2026 g. in 100 c.c.) was added. The readings are given in Table I (C). For these, 1 c.c. of solution was withdrawn at the times stated. A graph was then constructed, and the following 4 points were selected

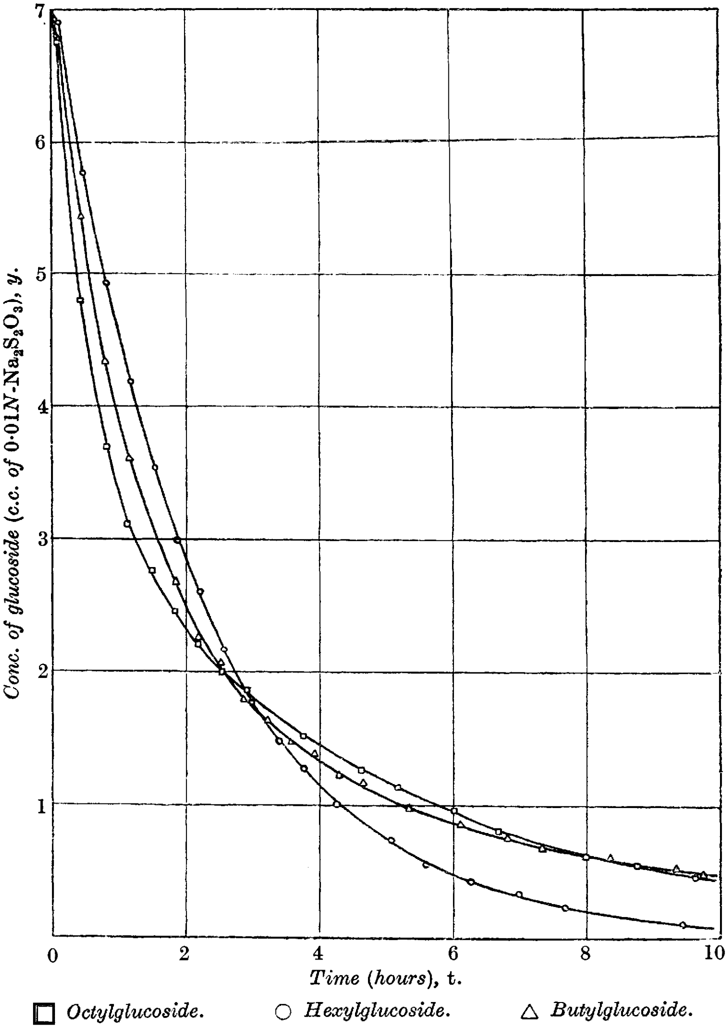
t (hours)	0	2	4	6
y	5·255	2·045	1·175	0·790

Preliminary exponential analysis gave the values

$$\bar{a}_1 = 2{\cdot}152 \qquad k_1 = 0{\cdot}170$$
$$\bar{a}_2 = 3{\cdot}103 \qquad k_2 = 0{\cdot}899$$

and the following equation was obtained after the usual correction :

$$y = 2{\cdot}16e^{-0{\cdot}166t} + 3{\cdot}08e^{-0{\cdot}909t}$$



Hence $k_2/k_1 = 5{\cdot}5$. The agreement between the mean curve and the calculated values is shown in Table II (C).

The experimental values are plotted in the figure.

The ratios of the reaction constants are thus 8·6, 1·5, and 5·5 for the octyl-, hexyl-, and butyl-glucosides respectively, so that under the conditions described the alkyl radical has a very considerable effect. It has been shown previously that with the octylglucosides the *d*-alkyl component is hydrolysed more quickly than the *l*-alkyl component. To find whether the same holds for the butylglucosides, a hydrolysis experiment was carried out with glucoside prepared from

TABLE I.

A. Octylglucoside.			B. Hexylglucoside.			C. Butylglucoside.		
Time, mins.	T_n.	$T_\infty - T_n$.	Time, mins.	T_n.	$T_\infty - T_n$.	Time, mins.	T_n.	$T_\infty - T_n$.
0	0·00	6·98	0	0·00	7·08	0	0·00	7·015
5·0	0·24	6·74	6·0	0·18	6·90	6·5	0·25	6·765
26	2·185	4·795	28·5	1·31	5·77	27	1·58	5·435
48·5	3·29	3·69	49·5	2·15	4·93	48	2·675	4·340
68·5	3·875	3·105	71	2·895	4·185	69·5	3·41	3·605
90·5	4·22	2·76	92	3·545	3·535	111·5	4·34	2·675
111	4·53	2·45	113	4·09	2·99	132·5	4·76	2·255
132	4·77	2·21	134	4·48	2·60	152·5	4·95	2·065
153	4·98	2·00	155	4·915	2·165	172·5	5·225	1·790
176	5·115	1·865	179·5	5·305	1·775	194·5	5·38	1·635
227·5	5·465	1·515	204	5·60	1·48	215	5·54	1·475
278	5·715	1·265	226	5·705	1·275	236·5	5·635	1·380
311	5·84	1·14	305	6·34	0·74	258	5·79	1·225
362	6·02	0·96	336	6·525	0·555	321	6·04	0·975
401	6·17	0·81	376	6·65	0·43	366	6·16	0·855
479	6·355	0·625	419	6·735	0·345	409	6·258	0·757
525	6·42	0·56	460·5	6·845	0·235	440·5	6·337	0·678
577	6·51	0·47	565·5	6·975	0·105	501	6·405	0·610
∞	6·98	0·00	∞	7·08	0·00	560	6·485	0·530
						584	6·533	0·482
						∞	7·015	0·00

TABLE II.

	A. Octylglucoside.			B. Hexylglucoside.			C. Butylglucoside.		
Time from P, hrs.	y (curve).	y (calc.).	Diff.	y (curve).	y (calc.).	Diff.	y (curve).	y (calc.).	Diff.
	5·21	5·18	+0·03	6·90	6·92	−0·02	6·77	6·74	+0·03
0	4·79	4·78	+0·01	5·43	5·46	−0·03	5·25	5·24	+0·01
0·5	3·43	3·47	−0·04	4·33	4·32	+0·01	3·91	3·94	−0·03
1	2·80	2·79	+0·01	3·42	3·42	0·00	3·07	3·07	0·00
1·5	2·39	2·39	0·00	2·71	2·72	−0·01	2·46	2·47	−0·01
2	2·08	2·08	0·00	2·15	2·16	−0·01	2·05	2·05	0·00
2·5	1·85	1·86	−0·01	1·70	1·73	−0·03	1·74	1·74	0·00
3	1·64	1·66	−0·02	1·37	1·38	−0·01	1·51	1·51	0·00
4	1·32	1·33	−0·01	0·89	0·89	0·00	1·17	1·19	−0·02
5	1·07	1·08	−0·01	0·58	0·58	0·00	0·95	0·98	−0·03
6	0·87	0·87	0·00	0·38	0·37	+0·01	0·79	0·81	−0·02
7	0·70	0·70	0·00	0·25	0·24	+0·01	0·68	0·68	0·00
8	0·58	0·57	+0·01	0·16	0·16	0·00	0·59	0·58	+0·01
9	0·49	0·46	+0·03	0·10	0·11	−0·01	0·51	0·49	+0·02

The time values for the first row are −0·1, −0·5, −0·4 for A, B, C, respectively, taken from P as zero.

a quantity of alcohol which had been partially resolved. From this it was found that the *l*-alkyl component was the one more quickly hydrolysed in this case.

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