CXCII.—*The Velocity of Hydrolysis of Starch and Maltose by Cold Concentrated and Fuming Hydrochloric Acid.*

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THE velocity of hydrolysis of starch and maltose by cold concentrated and fuming hydrochloric acid has been studied polarimetrically at 20.00°; the results obtained throw an interesting light on the nature of the changes occurring during the hydrolysis of polysaccharides, and afford confirmation of the views put forward by Davis (*J. Soc. Dyers*, 1914, **30**, 249) to explain certain abnormalities in the coefficient showing the velocity of action of enzymes on starch and maltose.

The great change of rotatory power which occurs when dextrose is dissolved in fuming hydrochloric acid is doubtless due to the transformation of β -glucose ($[\alpha]_D$ 19°) (which constitutes about 63 per cent. of the equilibrium mixture existing in aqueous solution) into α -glucose ($[\alpha]_D$ 110°). Similarly, if the initial specific rotatory power of maltose in fuming hydrochloric acid is calculated from the curves given on p. 2071, before any hydrolysis has taken place, a value of $[\alpha]_D^{20.0}$ 157.4° is obtained for acid of D^{15} 1.201; for the ordinary concentrated acid in a similar manner a value $[\alpha]_D^{20.0}$ 141.0° is obtained. When maltose is dissolved in water it shows mutarotation upwards, and after a short time equilibrium is established when $[\alpha]_D^{20.0}$ = 137.6°; this solution probably contains a mixture of a β -glucose- α -glucoside ($\beta\alpha$ -maltose) with $[\alpha]_D$ 114° and α -glucose- α -glucoside ($\alpha\alpha$ -maltose) with $[\alpha]_D$ as yet unknown, but probably higher than 160°. The result of dissolving maltose in fuming hydrochloric acid is to shift the equilibrium existing between these two forms so as largely to increase the proportion of the $\alpha\alpha$ -maltose of high rotatory power.

It has been suggested recently (Davis, *loc. cit.*) that the hydro-

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lysis of polysaccharides, for example, starch and maltose, by enzymes, does not follow the simple unimolecular law of mass action, because the action occurring is not a single change, but is made up of a series of successive changes. Thus in the case of maltose three changes at least occur:

- (1) $\beta\alpha$ -maltose $\rightarrow \alpha\alpha$ -maltose.
- (2) $\alpha\alpha$ -maltose $\rightarrow$ 2 mols. α -glucose.
- (3) α -glucose $\rightarrow$ equilibrium mixture of α - and β -glucose.

The velocity of the main change (2), which is brought about by the enzyme, will, as a mass action, be modified by the velocity with which changes (1) and (3) occur; in the faintly acid or nearly neutral solutions in which enzyme actions take place, these changes take an appreciable time, so that the velocity-coefficient of the main change (2), when calculated by the ordinary unimolecular equation, is not a constant, but undergoes considerable variation during the course of the change.

When fuming hydrochloric acid is used to effect the hydrolysis of maltose the case is different. The greater part of the $\beta\alpha$ -maltose is converted almost instantly into the form of higher rotatory power, so that immediately after the solution is made up the rotatory power has reached the high value $[\alpha]_D^{20.0} 157.4^\circ$.

After the hydrolysis of $\alpha\alpha$ -maltose is complete the α -glucose remains largely or completely in the α -form, without undergoing change into the β -form. The consequence is that the disturbing effect of the changes (1) and (3) on the velocity of the main change is largely eliminated; this is made evident by the fact that during the whole of the hydrolysis of maltose by fuming acid the velocity-coefficient is practically a constant, varying only from 0.0103 to 0.0106, a variation which can be accounted for by experimental error. In this case a single change only occurs.

With ordinary hydrochloric acid, however, in which maltose dissolves as an equilibrium mixture not very different from that existing in aqueous solution ($[\alpha]_D^{20.0} 141^\circ$ instead of 137.6°), the effect of equation (1) on the velocity-coefficient of the total change becomes distinctly visible. A steady rise in the velocity-coefficient occurs, namely, from 0.00170 to 0.00186, a variation of about 10 per cent.

When the hydrolysis of starch by acid is considered, the series of changes is more complex. In order to avoid the difficulty that arises with ordinary starch owing to the appreciable time that elapses before the solid passes completely into solution, soluble starch was used throughout. Even when soluble starch is the starting point, several stages have to be passed through on the way to dextrose; dextrans and maltose are formed as intermediaries.

The result is that in the case of fuming hydrochloric acid the velocity-coefficient changes to a much greater extent than even with maltose and ordinary hydrochloric acid. There is a steady rise from 0.0052 to 0.0063, a range of variation of 23 per cent. With ordinary concentrated hydrochloric acid the variation of the coefficient is still greater, namely, from 0.00111 to 0.00144; the range is 30 per cent.

The transformation of cellulose into dextrose studied by Willstätter and Zechmeister (*Ber.*, 1913, **46**, 2401) affords an extreme instance of the departure of the law of action from the ordinary unimolecular law, owing to the occurrence of successive actions. On p. 2072 is given a table showing the results obtained by calculating from Willstätter and Zechmeister's data the value of the ordinary velocity-coefficient $K = \log a/a - x$. Fig. 1 shows the velocity curve. During the earlier period (the first two hundred minutes) this exhibits a double inflexion, and exactly corresponds in character with the curve of hydrolysis of starch to dextrose by means of taka-diastase (Davis and Daish, *J. Agric. Sci.*, 1914, **6**, 152); as in the latter case, the velocity-coefficient calculated for a unimolecular equation at first rises and then falls (Davis, *loc. cit.*). During this period the cellulose is transformed into a soluble polysaccharide, having no rotatory power, then into dextrin-like compounds, and finally into cellobiose; this substance, as shown by the experiments made with it by Willstätter and Zechmeister, is relatively slowly attacked by fuming hydrochloric acid. The latter part of the curve in Fig. 1 (from two hundred and fifty minutes to three thousand one hundred minutes) probably represents mainly the transformation of this sugar into dextrose; it is nearly logarithmic; but during the period 205—450 minutes, cellobiose is still being formed from the cellulose, so that there is a reinforcing effect on the ordinary mass action, and the coefficient rises from 0.000401 to 0.000536. Subsequently it becomes more constant for the rest of the transformation.

We have then, exemplified in the action of fuming and concentrated hydrochloric acids on starch and cellulose, the result of a series of successive and overlapping changes, the mathematical analysis of which would be of a highly complicated character. These changes show very great similarity to many cases of enzyme action, and justify the view put forward by Barendrecht (*Biochem. J.*, 1913, **7**, 548) and Davis (*loc. cit.*) that the departure of the law of action of enzymes in many cases from a simple unimolecular equation is due to the fact that in such cases a series of changes really occurs. When the subsidiary changes, which usually give rise to mutarotation effects, can, as in the case of

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fuming acid and maltose, either be suppressed or rendered extremely rapid relatively to the main change, the action more and more closely approximates to the simple unimolecular law.

EXPERIMENTAL.

Action of Fuming Hydrochloric Acid on Maltose.

1.0555 Grams of carefully purified anhydrous maltose (dried in a vacuum at 105°, $[\alpha]_D^{20}$ 137.6° in 1 per cent. aqueous solution) were dissolved in fuming hydrochloric acid (D¹⁵ 1.201); the volume was made up to 50 c.c. at 20°, and the solution examined in a 220-mm. tube at 20.00°.

When the velocity-coefficient is calculated from the ordinary unimolecular equation $K = 1/t \log_{10} a/a-x$, values ranging from 0.0094 to 0.0101 are obtained, the mean value being 0.0098.

This equation necessitates the use of an initial value corresponding with time $t=0$; this was obtained as accurately as was possible by continuing the curve (Fig. 2) backwards, so as to cut the axis of t . As emphasised by Worley (T., 1911, **99**, 352), any error in this initial value affects the accuracy of the whole series of values calculated for K . It is therefore preferable to calculate K from Worley's modified form of the equation:

$$K \log e = \frac{1}{t_2 - t_1} \log_{10} \frac{a - x_1}{a - x_2},$$

where $a - x_1$ and $a - x_2$ are the concentrations of sugar at times t_1 and t_2 respectively, the successive intervals of time being taken as small as the accuracy of the observations will allow or the rapidity of the change demands. This has been done in table I.

TABLE I.

Action of Fuming Hydrochloric Acid on Maltose at 20.00°.

Time in minutes.	a_t .	$a_t - a_{\infty}$.	$K[\log e]$.
0	—	—	—
10	6.85	2.16	—
15	6.63	1.94	—
20	6.43	1.74	—
25	6.24	1.55	—
30	6.07	1.38	—
35	5.90	1.21	—
40	5.75	1.06	0.0103
45	5.63	0.94	0.0105
50	5.53	0.84	0.0105
55	5.44	0.75	0.0105
60	5.36	0.67	0.0105
65	5.28	0.59	0.0104
70	5.20	0.51	0.0106
75	5.14	0.45	0.0106
80	5.08	0.39	{0.0111}
85	5.02	0.33	—
90	4.96	0.27	—
Final	4.69	—	—
Mean			0.0105

The values obtained for K by this method of calculation are probably more accurate than those obtained from the ordinary equation. It is seen that within the limits of experimental error the value of $K \log e$ is constant.

Action of Ordinary Concentrated Hydrochloric Acid on Maltose.

1.0555 Grams of anhydrous maltose were dissolved in concentrated hydrochloric acid ($D^{15} 1.163$), and the volume was made up to 50 c.c. at 20° . The solution was examined in a 220-mm. tube at 20.00° (Fig. 1).

FIG. 1.

Action of concentrated hydrochloric acid ($\Delta=1.163$) on starch and maltose at 20° .

Calculating the value of $K \log e$ by Worley's equation we have the following values for the velocity-coefficient.

TABLE II.

Action of Concentrated Hydrochloric Acid on Maltose at 20.00° .

Time in minutes.	α_t	$\alpha_t - \alpha_\infty$	$K \log e$
0	—	—	—
50	5.97°	2.758°	—
100	5.49	2.278	—
150	5.09	1.878	—
200	4.76	1.548	—
250	4.47	1.258	(0.00170)
300	4.23	1.018	0.00174
350	4.04	0.828	0.00178
400	3.87	0.658	0.00186
450	3.75	0.538	0.00184
500	3.65	0.438	0.00183
550	3.57	0.358	0.00182
600	3.50	0.288	0.00179
650	3.44	0.228	0.00186
Final	3.212	—	—
		Mean	0.00180

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The velocity-coefficient is clearly not constant. There is a steady rise in the value as time proceeds. For comparison, however, with the results with the fuming acid a mean value of K has been calculated. It is nearly constant during the latter stages of the action.

Action of Fuming Hydrochloric Acid on Soluble Starch.

1.000 Gram of soluble starch ($[\alpha]_D^{20.0}$ 198°, $c=2$; $R=1.0$) dried in a vacuum at 130° was dissolved in concentrated hydrochloric acid (D¹⁵ 1.201), and the volume made up to 50 c.c. at 20.0°; the solution was examined in a 220-mm. tube at 20.0° (curve, Fig. 2).

TABLE III.

Action of Fuming Hydrochloric Acid on Soluble Starch at 20.00°.

Time in minutes.	α_t .	$\alpha_t - \alpha_\infty$.	$K \log e$.
0	—	—	—
5	10.20°	5.51°	—
10	9.90	5.21	—
15	9.61	4.92	—
20	9.32	4.63	—
25	9.05	4.36	—
30	8.78	4.09	—
35	8.54	3.85	0.0052
40	8.30	3.61	0.0053
45	8.07	3.38	0.0054
50	7.85	3.16	0.0055
55	7.64	2.95	0.0057
60	7.43	2.74	0.0058
65	7.24	2.55	0.0060
70	7.06	2.37	0.0061
75	6.89	2.20	0.0062
80	6.73	2.04	0.0063
85	6.59	1.90	0.0064
90	6.46	1.77	0.0063
Final	4.69	—	—

In the case of soluble starch the velocity-coefficient is not constant, but rises continuously throughout the hydrolysis. The range of variation is about 20 per cent.

Action of Concentrated Hydrochloric Acid on Soluble Starch.

2.000 Grams of anhydrous soluble starch were dissolved in concentrated hydrochloric acid (D 1.163), and the volume was made up to 100 c.c. at 20.0. The solution was examined in a 220-mm. tube at 20.0° (Fig. 3).

FIG. 2.

Action of fuming hydrochloric acid ($\Delta = 1.201$) on starch and maltose at 20° .

FIG. 3.

Action of fuming hydrochloric acid ($\Delta = 1.210$) on cellulose (Willstätter and Zechmeister).

TABLE IV.

Concentrated Hydrochloric Acid on Soluble Starch at 20·0°.

Time in minutes.	a_t .	$a_t - a_\infty$.	$K \log e$.
0	—	—	—
50	8·34°	5·13°	—
100	7·80	4·59	—
150	7·25	4·04	—
200	6·75	3·54	—
250	6·29	3·08	0·00111
300	5·86	2·65	0·00119
350	5·47	2·26	0·00126
400	5·09	1·88	0·00137
450	4·80	1·59	0·00143
500	4·57	1·36	0·00145
550	4·37	1·16	0·00145
600	4·20	0·99	0·00139
650	4·05	0·84	0·00139
700	3·92	0·71	0·00141
750	3·80	0·59	0·00147
800	3·72	0·51	0·00144
Final	3·21	—	—

The velocity-coefficient rises continuously throughout the change, the range being nearly 30 per cent.

TABLE V.

Action of Fuming Hydrochloric Acid on Cellulose (Fig. 3).

Velocity of hydrolysis for the whole action, calculated for the simple unimolecular equation, from Willstätter and Zechmeister's data.

Time in minutes.	a_t .	$a_t - a_\infty$.	$K = \frac{1}{t} \log \frac{a_0 - a_\infty}{a_t - a_\infty} \times 10^6$.
0	0·00°	1·97°	—
40	0·02	1·95	113
105	0·13	1·84	283
115	0·18	1·79	362
130	0·25	1·72	461
145	0·27	1·70	442
155	0·28	1·69	430
170	0·29	1·68	407
185	0·29	1·68	374
205	0·34	1·63	401
255	0·47	1·50	464
450	0·84	1·13	536
480	0·89	1·08	544
515	0·93	1·04	539
575	1·02	0·95	551
1665	1·68	0·29	500
1925	1·76	0·21	505
3100	1·97	—	—

The velocity-coefficient rises to a maximum, then falls, and again rises and again falls.

TABLE VI.

Action of Fuming Hydrochloric Acid on Cellulose.

Velocity of hydrolysis during latter part of action, calculated by means of Worley's equation.

Time in minutes.	α_t (from curve).	$\alpha_t - \alpha_{\infty}$.	$K \log e \times 10^6$.
200	0.335°	1.635°	—
300	0.570	1.400	—
400	0.760	1.210	—
500	0.915	1.055	—
600	1.040	0.930	—
700	1.140	0.830	—
800	1.225	0.745	—
900	1.300	0.670	—
1000	1.370	0.600	544
1100	1.430	0.540	517
1200	1.490	0.480	502
1300	1.540	0.430	487
1400	1.583	0.387	476
1500	1.624	0.346	475
1600	1.660	0.310	476
1700	1.691	0.279	476
1800	1.724	0.246	484
1900	1.751	0.219	490
Final	1.970	—	—

The velocity-coefficient at first falls rapidly and then again rises.

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