LXX. THE HYDROLYSIS OF ASYMMETRIC ESTERS BY LIPASE.

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THE selective hydrolysis of racemic esters was demonstrated by Dakin [1904], but the esters employed by him belonged mainly to the acids and alcohols of the aromatic series. Little work has been carried out on the hydrolysis of asymmetric aliphatic esters. Dakin's results, obtained with an extract of liver as a source of lipase, have recently been confirmed and extended to the action of lipase from various sources by Willstätter and his collaborators. It is interesting to find that Willstätter and Memmen [1924] regard pancreatic lipase as having a greater specificity than liver lipase, even when both enzymes select the same enantiomorph for hydrolysis. In many of the racemic mixtures examined, however, these investigators find that the liver lipase hydrolyses a different enantiomorph from that attacked by the pancreatic enzyme. We find that this difference between the actions of liver and pancreatic lipase holds when tartaric esters are used as substrates.

Abderhalden, Sickel and Ueda [1923] have observed preferential hydrolysis of *l*-tyrosine ethyl ester in a racemic mixture. The fact that in racemic leucine esters the laevo-component is more easily hydrolysed has been demonstrated by Rona and Spiedel [1924] and also by Willstätter and Memmen [1924]. The hydrolysis of this ester is definitely attributed to lipase and not to a proteolytic enzyme as claimed by Warburg [1906]. No definite results were obtained by Dakin [1905] using aliphatic esters as substrates, owing to practical difficulties, coupled with the small rotations of these esters. No evidence of asymmetric hydrolysis, by a lipase of cow's pancreas, of ethyl, amyl and capryl esters of *dl*-lactic acid could be obtained by Murachi [1925].

Using an active preparation of pancreatic lipase and maintaining a suitable hydrogen ion concentration by the addition of buffer mixtures [Platt and Dawson, 1925] we have been able to obtain definite evidence of preferential hydrolysis in racemic mixtures of some aliphatic esters. Further, in the case of the tartaric esters we have confirmed the results obtained by polarimetric measurements by direct comparison of the amounts of hydrolysis of the stereoisomers.

EXPERIMENTAL.

The asymmetric derivatives of acetic acid were prepared by the action of alkyl halides on sodium malonic ester by the well-known method. It may be added that in the case of the *p*-nitrobenzylethyl derivative, Lellmann and Schleich [1887], who first prepared it, failed to obtain the intermediate malonic acid derivative. This was obtained by allowing *p*-nitrobenzylethylmalonic ester (12 g.) to stand with an alcoholic solution of potassium hydroxide (4.5 g. in 4.5 cc. water and 25 cc. alcohol) in the cold for 10 days. The acid which was separated (6 g.) melted at 102–105°, and gave off carbon dioxide on heating *in vacuo* at 130°. The product (5 g.) gave the correct melting point for *p*-nitrobenzylethylacetic acid, m.p. 43–45°. The ethyl ester crystallises in large colourless prisms from light petroleum, m.p. 41–42°. For the *iso*-butyl *d*- and *l*-tartrates and the *l*-tartaric acid we are indebted to Prof. T. S. Patterson of Glasgow University.

In preliminary tests we found that the hydrolysis occurring in the presence of lipase, though often effected with difficulty, was due to the enzyme, except in the case of the ethyl tartrates, where part of the hydrolysis was caused by the buffer solution alone. The amount of enzyme used for each estimation was relatively large, 0.5 g., half being added initially and the remainder at an intermediate stage in the reaction period. Phosphate buffer solutions at p_H 7.0 were used, of such a concentration (0.05 *M*) as to maintain throughout the course of hydrolysis a p_H suitable for the lipase action. The reaction mixtures were kept for several days at 37°, except in the case of Exps. 11 and 14. In the former experiment the ester, *p*-nitrobenzylethylacetic ethyl ester, was kept at 44°, a temperature above its melting point, and in Exp. 14 the temperature was lowered to 22° in an attempt to minimise the hydrolysis of the ethyl racemate by a buffer solution alone. A sample was taken from each mixture and the amount of hydrolysis determined by titration with standard sodium carbonate solution. The values given in the table are ex-

Exp.	Ethyl ester of	Temp. ° C.	Amount of ester g.	Amount of buffer cc.	Dura- tion of exp. days	Hydro- lysis of ester %	Sign of rotation of un- changed ester
1	Methylethylacetic acid	37	8.5	85	6	20	—
2	" "	"	4.4	105	6	41	—
3	Methylbutylacetic acid	"	8.5	85	6	16.5	—
4	" "	"	4.4	105	6	33	—
5	Ethylbutylacetic acid	"	8.5	85	6	18	Nil
6	" "	"	4.4	105	6	36	"
7	Methylbenzylacetic acid	"	5.0	100	5	45	—
8	" "	"	3.0	50	3	35	—
9	Ethylbenzylacetic acid	"	5.0	100	3	15	Nil
10	" "	"	5.0	50	6	14	"
11	<i>p</i> -Nitrobenzylethylacetic acid	44	4.75	50	8	30	"
12	Allylbenzylacetic acid	37	5.0	50	6	13	"
13	" "	"	5.0	75	4	15	"
14	Racemic acid	22	12.0	100	6	7.5	+
15	"	37	12.0	100	4	7.0	+

pressed as the percentage of the ester hydrolysed. In order to extract the unchanged ester, the calculated amount of sodium carbonate solution was added and the mixture repeatedly extracted with large volumes of ether. The ether was removed and the rotation, if any, of the residual ester in 50 % alcohol solution was determined polarimetrically. No figures are given for the amounts of rotation as the results have only a qualitative significance.

It will be seen that the diethyl *l*-tartrate is more readily hydrolysed than the dextro-ester in a racemic mixture. The rate of hydrolysis by lipase of diethyl racemate was found to be greater than that of diethyl *d*-tartrate. To prove that this difference is due to the more rapid liberation of *l*-tartaric acid from the racemate, we compared the speeds of hydrolysis of *d*- and *l*-diethyl tartrates. A typical result (Exp. 16) shows clearly that the ester of the laevo-acid is more rapidly hydrolysed.

Exp. 16. Mixtures were made up in flasks so as to contain 0.5 cc. ester (0.6 g. of the *i*-tartrate), 25 cc. phosphate buffer solution, p_H 7.0, and 50 mg. of enzyme. These were shaken in an incubator at 37° and the amount of hydrolysis at the end of 24 hours was determined by titration. The results are expressed in cc. of *N*/10 NaOH required to neutralise the acid formed and are the means of duplicate experiments corrected for the necessary controls.

Substrate	Mean titration value (corr.) cc.
Diethyl ester of <i>d</i> -tartaric acid	1.00
„ <i>l</i> -tartaric acid	4.77
„ racemic acid	2.89
„ <i>i</i> -tartaric acid	0.63

The diethyl *i*-tartrate was hydrolysed at approximately the same rate as the ester of *d*-tartaric acid.

The same difference between the rates of hydrolysis of stereoisomers was also demonstrated with the *iso*-butyl esters of *d*- and *l*-tartaric acids.

Exp. 17. 0.25 g. of ester used, other conditions as in Exp. 16.

Substrate	Mean titration value (corr.) cc.
<i>iso</i> -Butyl <i>d</i> -tartrate	1.11
<i>iso</i> -Butyl <i>l</i> -tartrate	9.10

The hydrolysis of tartaric esters by liver lipase.

The enzyme used was obtained as a powder by drying a press-juice from ox-liver with acetone and ether.

Exp. 18. 200 mg. of liver powder were added to

- 0.75 cc. of diethyl tartrate in 25 cc. of phosphate buffer solution, p_H 7.0.
- 0.1 g. of *iso*-butyl tartrate in 10 cc. of the same buffer solution.

These mixtures were incubated for 24 hours and the amount of hydrolysis estimated as in Exp. 16.

Substrate	Mean titration value (corr.) cc.
Diethyl <i>d</i> -tartrate	7.45
Diethyl racemate	4.60
<i>iso</i> -Butyl <i>d</i> -tartrate	5.3
<i>iso</i> -Butyl <i>l</i> -tartrate	4.6

Liver lipase, therefore, unlike pancreatic lipase, hydrolyses the esters of *d*-tartaric acid more readily than those of the *l*-acid.

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