to which 25 cc. of hydrocyanic solution and 2 cc. benzaldehyde were added. After $3^1/_2$ hours, the usual extraction was effected and the nitrile hydrolyzed with hydrochloric acid. The hydrochloric acid solution (50 cc.) showed a rotation of —1.45° in a 2 dcm. tube. The presence of the glucose, therefore, did not change the activity from dextro to levo.

Another experiment was carried out similar to the one above only substituting for glucose *l*-amygdalin, but the resulting mandelic acid was levo active.

Investigations will be continued on this subject to discover if possible the cause for the production of these two nitriles. The barks and leaves of wild cherry and elder berry will also be extracted to see if there is any difference in the emulsin produced.

In conclusion, I wish to thank Dr. Walker for proposing this investigation and for his suggestions and interest during its progress; also Dr. McIntosh for many practical suggestions.

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STUDIES ON ENZYME ACTION. I. SOME EXPERIMENTS WITH THE CASTOR BEAN LIPASE.

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This investigation was undertaken with the object of studying the action of a fat or ester splitting ferment (lipase) more particularly from the point of view of the ferment itself and its behavior under varying conditions. The lipase present in castor beans was chosen as most suitable for the present work.

The lipase preparation used in the experiments was prepared as follows: The castor beans[1] were ground roughly, extracted a number of times with carbon tetrachloride or chloroform,[2] then ground to a fine powder and passed through a 100-mesh sifter. In this way a large portion of the shells were separated. The fine powder was then extracted exhaustively with ether (100 times or more) in a Soxhlet extractor, practically all of the fat being removed in this way.[3] The following experiment shows the activity of the preparation at the different stages: 2 cc. olive oil and 50 cc. water were treated with the substances stated for 17 hours at 38° and the acid present then titrated with a 0.1 *N* sodium hydroxide solution, using phenolphthalein as indicator. No castor bean present = 0.6 cc. alkali required; 0.2 gram castor beans before sifting = 1.0 cc.; 0.2 gram castor beans after sifting (shells removed) = 1.9 cc.; 0.2 gram

[1] The castor beans were supplied by the Baker Castor Oil Company of New York.

[2] This extraction was carried out by Mr. C. W. Otto.

[3] Cf. A. E. Taylor, *J. Biol. Chem.*, **2**, 87 (1906), for the preparation of the lipase material from castor beans and a careful study of its properties.

castor beans after sifting and extraction with ether = 1.7 cc. This gives an approximate idea of the activity of the preparation. The olive oil used here was itself slightly acid in reaction and became more so on standing at the temperature in question in contact with water. The removal of the shells increased the activity markedly for the same weight of substance, as the shells do not contain the active constituent,[1] while the extraction with ether had very little effect, the slight decrease probably being due to the solubility of lipase in ether containing a fatty body, as Taylor pointed out.

In the experiments to be described, unless expressly stated to the contrary, no substance was added to the lipase mixture to increase the activity, either to form a more stable emulsion, increase the solubility, or act as activator.[2] No attempts were made to find the conditions for maximum action. The hydrolyses obtained are therefore, as a rule, smaller in magnitude than those found by others. Blank experiments were carried out with each series of experiments with lipase and ester, using lipase (and solvent) alone and ester (and solvent) alone; in this way corrections were introduced and the action of the lipase on the ester alone could be more closely approximated, although an uncertainty may be introduced by the possibility of a more complex action between lipase, ester and solvent aside from the hydrolytic action, compared with the sum of the actions of lipase and solvent and ester and solvent used as correction. The amount of ester hydrolyzed was measured in every case by titrating with a 0.1 *N* aqueous solution of sodium hydroxide, using 3–5 drops of a 1% solution of phenolphthalein in 95% ethyl alcohol as indicator and titrating to a distinct pink color. Owing to the presence of the solid suspension (lipase preparation), the end-point was sometimes difficult to determin, but in general the titrations were accurate to 0.1 cc. and in some cases where particular difficulty was encountered, to 0.2 cc.

The castor bean lipase preparation as used in the following experiments still contained a small proportion of shells. The preparation was light gray in color and on ignition gave a residue of white ash amounting to 9%. The preparation was slightly acid in reaction when mixed with water, 0.2 gram requiring 0.2 cc. 0.1 *N* sodium hydroxide solution for neutrality to be attained. The acidity increased slowly on standing, but whether this was due to a minute quantity of oil which had not been removed by the ether and which was slowly hydrolyzed, or to the slow

[1] Nicloux, *Mem. Soc. Biol.*, 56, 701, 839, 868 (1904).

[2] Cf. Rosenheim and Shaw-Mackenzie, *Proc. Lond. J. Physiol*, 40, I, II, III, Feb., 1910, for the effect of emulsification with pancreas lipase and for the action of activators. Also Armstrong and Ormerod, *Proc. Roy. Soc.*, 78, 376 (1906), and others. Recently, Jalander, *Biochem. Z.*, 36, 437 (1911), followed the formation of the lipase-fat emulsion with the microscope.

decomposition of some of the substances present in the lipase preparation itself with the formation of acid, cannot be stated. In view of the long continued extraction with ether, the balance of probability would appear to rest with the latter view.

The general outline of the experiments performed for studying the hydrolytic action of the lipase preparation was as follows: Three esters were studied: methyl acetate, an example of a simple ester soluble in water; ethyl butyrate, which has been extensively used in investigations of this kind; and olive oil, as a substance closely related to the fats. After some comparative experiments in aqueous solution (experiments 1–12 in Table I), a number of experiments were carried out with different amounts of alkali added initially to determin what action, if any, the small amount of acid always present in the lipase preparation exerted (experiments 13–30). To test this point further, measurements were also made in moist ether, and acetone containing a small amount of water, both at the ordinary and at somewhat higher temperature (experiments 32–53). No experiment was allowed to last for more than 50 hours on account of the possibility of complicating the reaction by the continual increase in acidity of the lipase preparation itself.

Table I summarizes the results obtained. The first column shows the number of the experiment. Each experiment consists of three separate measurements, the results of which are shown in columns 7, 8, and 9. The amounts of substances used shown in columns 2, 3, and 4 refer in each experiment to the three sets of measurements. The esters used (column 2) were methyl acetate—MeAc—obtained from Kahlbaum and redistilled after treatment with sodium carbonate; ethyl butyrate—EtBu—purified by washing the ethereal solution free from acid and subsequent redistillation; and olive oil, the purest obtainable, and unless stated to the contrary, neutral in reaction before the experiment. The solvents are shown in column 3, moist ether referring to ether saturated with water at room temperature. Columns 4, 5, and 6 refer respectively to the weights of lipase preparation used, the duration of the experiments and the temperatures maintained. Columns 7, 8, and 9 show the experimental results found for each set of measurements, first with lipase, ester, and solvent, and then with ester and solvent alone, and with lipase and solvent alone. The numbers refer to the number of cubic centimeters of a 0.1 *N* aqueous solution of sodium hydroxide required to produce a distinct pink color with phenolphthalein. For the experiments in which ether or acetone was used as solvent, 50 cc. of water were added before the titration and the solution thoroughly agitated after each addition of alkali. A satisfactory end-point was obtained in this way. Column 10 gives the difference between the results in column 7 and the sum of the results in columns 8 and 9; that is to say, the amount

TABLE I.—THE HYDROLYTIC ACTION OF THE CASTOR BEAN LIPASE.

Expt. No.	Ester.	Solvent.	Lipase. Gram.	Time. Hrs.	Temperature.	Action in cc. 0.1 *N* sodium hydroxide solution. Ester, lipase and solvent.	Ester and solvent.	Lipase and solvent.	Ester and lipase.
1.....	2 cc. MeAc	50 cc. water	0.2	17	38°	3.75	0.90	1.25	1.60
2.....	1 cc. MeAc	40 cc. water	0.2	23	38°	1.95	0.15	0.60	1.20
3.....	1 cc. MeAc	25 cc. water	0.2	42	20°	2.40	0.10	0.50	1.80
4.....	2 cc. MeAc	25 cc. water	0.5	42	20°	5.75	0.60	1.30	3.85
5.....	1 cc. EtBu	40 cc. water	0.2	23	38°	1.70	0.10	0.60	1.00
6.....	1 cc. EtBu	25 cc. water	0.2	50	38°	2.50	0.20	0.70	1.60
7.....	1 cc. EtBu	25 cc. water	0.2	50	20°	1.70	0.05	0.70	0.95
8.....	2 cc. EtBu	25 cc. water	0.2	48	20°	1.50	0.10	0.50	0.90
9.....	2 cc. EtBu	30 cc. water	1.0	48	20°	10.00	0.05	2.40	7.55
10.....	1 cc. olive oil	40 cc. water	0.2	23	38°	0.90	0.10	0.60	0.20
11.....	2 cc. olive oil	25 cc. water	0.5	42	20°	1.70	0.10	1.30	0.30
12.....	2 cc. olive oil	30 cc. water	1.0	48	20°	4.90	0.05	2.40	2.45
13.....	1 cc. MeAc	40 cc. water, 0.2 cc. 0.1 *N* NaOH	0.2	22	20°	1.40	0.10	0.15	1.15
14.....	1 cc. MeAc	25 cc. water, 0.2 cc. 0.1 *N* NaOH	0.2	42	20°	2.20	0.10	0.20	1.90
15.....	1 cc. MeAc	40 cc. water, 0.2 cc. 0.1 *N* NaOH	0.2	46	20°	2.00	0.10	0.20	1.70
16.....	1 cc. MeAc	40 cc. water, 0.2 cc. 0.1 *N* NaOH	0.2	22	38°	1.80	0.20	0.50	1.10
17.....	1 cc. MeAc	25 cc. water, 0.2 cc. 0.1 *N* NaOH	0.2	42	38°	2.30	0.30	0.90	1.10
18.....	1 cc. MeAc	40 cc. water, 0.2 cc. 0.1 *N* NaOH	0.2	46	38°	2.95	0.35	1.40	1.20
19.....	1 cc. MeAc	40 cc. water, 0.27 cc. 0.1 *N* NaOH	0.2	20	38°	1.75	0.20	0.40	1.15
20.....	1 cc. MeAc	40 cc. water, 0.27 cc. 0.1 *N* NaOH	0.2	44	38°	2.50	0.30	0.50	1.70
21.....	1 c . MeAc	40 cc. water, 0.45 cc. 0.1 *N* NaOH	0.2	20	38°	1.65	0.30	0.30	1.05
22.....	1 cc. MeA	40 cc. water, 0.45 cc. 0.1 *N* NaOH	0.2	44	38°	2.40	0.35	0.35	1.70
23.....	1 cc. EtBu	40 cc. water, 0.2 cc. 0.1 *N* NaOH	0.2	22	20°	0.75	0.05	0.20	0.50
24.....	1 cc. EtBu	25 cc. water, 0.2 cc. 0.1 *N* NaOH	0.2	50	20°	1.50	0.05	0.50	0.95

25.....	1 cc. EtBu	40 cc. water, 0.2 cc. 0.1 *N* NaOH	0.2	22	38°	1.45	0.10	0.50	0.85
26.....	1 cc. EtBu	40 cc. water, 0.2 cc. 0.1 *N* NaOH	0.2	46	38°	2.10	0.10	1.40	0.60
27.....	1 cc. EtBu	40 cc. water, 0.27 cc. 0.1 *N* NaOH	0.2	20	38°	1.20	0.10	0.40	0.70
28.....	1 cc. EtBu	40 cc. water, 0.27 cc. 0.1 *N* NaOH	0.2	44	38°	1.55	0.20	0.50	0.85
29.....	1 cc. EtBu	40 cc. water, 0.45 cc. 0.1 *N* NaOH	0.2	20	38°	0.95	0.30	0.15	0.50
30.....	1 cc. EtBu	40 cc. water, 0.45 cc. 0.1 *N* NaOH	0.2	44	38°	1.10	0.20	0.35	0.55
31.....	1 cc. EtBu	25 cc. water, 0.2 cc. 0.12 *N* HCl	0.2	50	38°	3.20	1.20	0.70	1.30
32.....	1 cc. olive oil	40 cc. water, 0.2 cc. 0.1 *N* NaOH	0.2	22	20°	0.20	0.05	0.20	—0.05
33.....	1 cc. olive oil	40 cc. water, 0.2 cc. 0.1 *N* NaOH	0.2	46	20°	0.30	0.05	0.20	0.05
34.....	1 cc. olive oil	40 cc. water, 0.2 cc. 0.1 *N* NaOH	0.2	22	38°	0.80	0.10	0.50	0.20
35.....	1 cc. olive oil	40 cc. water, 0.2 cc. 0.1 *N* NaOH	0.2	46	38°	2.00	0.10	1.40	0.50
36.....	1 cc. olive oil	40 cc. water, 0.27 cc. 0.1 *N* NaOH	0.2	20	38°	0.55	0.10	0.40	0.05
37.....	1 cc. olive oil	40 cc. water, 0.27 cc. 0.1 *N* NaOH	0.2	44	38°	0.95	0.20	0.50	0.25
38.....	1 cc. olive oil	40 cc. water, 0.45 cc. 0.1 *N* NaOH	0.2	20	38°	0.45	0.10	0.30	0.05
39.....	1 cc. olive oil	40 cc. water, 0.45 cc. 0.1 *N* NaOH	0.2	44	38°	1.35	0.20	0.35	0.80
40.....	1 cc. MeAc	35 cc. moist ether	0.2	48	20°	1.40	0.20	0.60	0.60
41.....	2 cc. MeAc	40 cc. moist ether	1.0	48	20°	6.60	0.20	3.20	3.20
42.....	2 cc. MeAc	50 cc. moist ether	0.5	6¼	35–40°	2.90	0.20	1.10	1.60
43.....	1 cc. MeAc	24 cc. acetone, 1 cc. water	0.2	48	20°	0.80	0.20	0.60	0
44.....	2 cc. MeAc	24 cc. acetone, 1 cc. water	0.5	42	20°	2.80	0.50	1.20	1.10
45.....	2 cc. MeAc	48 cc. acetone, 2 cc. water	0.5	4	56–60°	2.70	0.30	1.80	0.60
46.....	2 cc. EtBu	35 cc. moist ether	0.2	48	20°	0.80	0.20	0.60	0
47.....	1 cc. EtBu	50 cc. moist ether	0.5	6	20°	1.70	0.30	1.40	0
48.....	1 cc. EtBu	50 cc. moist ether	0.5	6	35–40°	1.50	0.20	1.30	0
49.....	2 cc. EtBu	40 cc. moist ether	1.0	48	20°	3.90	0.20	3.20	0.50
50.....	2 cc. EtBu	24 cc. acetone, 1 cc. water	0.2	48	20°	0.65	0.15	0.60	—0.10
51.....	2 cc. EtBu	27 cc. acetone, 3 cc. water	1.0	48	20°	4.40	0.15	3.90	0.35
52.....	2 cc. olive oil	40 cc. moist ether	1.0	48	20°	3.50	0.20	3.20	0.10
53.....	2 cc. olive oil	24 cc. acetone, 1 cc. water	0.5	42	20°	2.70	1.10	1.20	0.40

of action[1] between the lipase and the ester in the production of acid after subtracting the changes which the lipase preparation alone and the ester alone had undergone in the presence of the solvent. A few drops of toluene were added to each solution as antiseptic.

Before discussing the results shown in the table in detail, some general remarks may be made. The correction due to the action between ester and solvent is as a rule small (omitting experiment 31 in which a hydrochloric acid solution was used as solvent), exceeding 0.2 cc. in 9 cases out of 52 and 0.35 cc. in only 2 (experiments 1 and 4) in which ester was used which was originally slightly acid. The correction due to the action between lipase and solvent is more variable. This correction was determined in every case with some of the same lipase preparation used with the ester and made up with some of the same solvent, and treated in an identical manner. In this way it was hoped to introduce a more exact correction than if an average change of the lipase preparation with the solvent had been taken from a large number of separate experiments. It must be stated, however, that in two or three experiments (not given in the table) the lipase preparation after standing in contact with the solvent showed a very large increase in acidity, such as would give a considerable negative value for the action between lipase and ester. This only occurred in isolated cases and never for both parts of duplicate experiments carried on simultaneously. This change must therefore be considered as out of the ordinary and due to some unknown reaction in the lipase preparation. It is not considered further here, but the possibility of this occasional large increase must be kept in mind in considering any apparently exceptional experiment, as will appear later.

In (1)[2] the ester, methyl acetate, and lipase were slightly acid in character initially, making the correction terms rather large. The corrected action between lipase and ester is somewhat larger than that found in (2) but a strict comparison is not permissible as different amounts of ester and solvent were used. The results of (3) and (4) call for no special remark. With ethyl butyrate (5–9) the amount of ester used exerts no influence (7,8), doubtless on account of its limited solubility, and, with an excess present, constant concentration in solution. There is considerably less action than with methyl acetate, but a comparison with these results is impracticable. This can, of course, only be done satisfactorily with equivalent amounts of ester wholly in solution. With a larger quantity of lipase (9) there is more than a proportionately greater

[1] Strictly speaking, to this should be added the amount of alkali necessary to produce the same pink color with phenolphthalein, as this color was produced twice with the experiments in columns 8 and 9, and once with the experiment in column 7. This correction was not introduced, as the experimental error doubtless exceeds it.

[2] The numbers in parentheses refer to the numbers of the experiments in Table I.

action. With olive oil (10–12) there is much less action than with the simple esters, probably on account of its slight solubility.

Since, in these experiments, the lipase preparation was slightly acid in character, attempts were made to eliminate this acidity and to follow the action, starting with a medium neutral toward phenolphthalein.[1] As stated before, 0.2 gram of the preparation required 0.2 cc. 0.1 *N* sodium hydroxide solution. Three series of experiments were carried out with each ester and 0.2 gram of lipase preparation; one in which 0.2 cc. 0.1 normal sodium hydroxide solution were added initially (13–18, 23–26, 32–35); one in which 0.27 cc. were added (19–20, 27–28, 36–37); and one in which 0.45 cc. were added (21–22, 29–30, 38–39). Results were obtained for two-time intervals; 20–24 hours and 42–50 hours.

With methyl acetate and 0.2 cc. alkali (13–15) at 20° there is considerably more action (in the ratio of 3–2) for the time interval of 42 or 46 hours than for 22 hours. At 38°, however (16–18) there is the same apparent action for the two-time intervals, although the correction for the lipase-solvent action appears rather large for the longer intervals. This will be referred to again presently. With 0.27 cc. (19, 20) and 0.45 cc. (21, 22) alkali the action is practically identical with that observed at 20° and 0.2 cc. alkali, the ratio of the amounts of action being about 3 : 2 for the two-time intervals. Including the results of experiments 2 and 3, for an experiment lasting 20–23 hours, the amount of action was found to be 1.05–1.20 cc. alkali at 20° or at 38° in the presence or absence of initially added alkali in amount up to 0.45 cc. 0.1 *N* sodium hydroxide solution.[2] For an experiment lasting 42–46 hours, the action was approximately 1.80 cc. at 20° with or without 0.2 cc. added alkali and at 38° with 0.27 cc. or 0.45 cc. added alkali. Schütz's rule that with the same amount of enzyme material, the amounts hydrolyzed are proportional to the square roots of the times of action is approximately followed here; amounts hydrolyzed 3 : 2; times of action 2 : 1. The fact that at 38°, 42–46 hours and with 0.2 cc. NaOH solution added initially (16–18), the experiments (17–18) show no difference as compared with the action for 22 hours, in view of the results just discussed, throws doubt on these (17–18), results, and as the lipase-solvent correction is markedly higher here, this must be ascribed to experimental error, and as pointed out in the general discussion, to the high value for the lipase-solvent correction. Experiments 26 and 35 were carried on simultaneously with the same lipase-solvent blank, and the results obtained with these must therefore also be viewed with caution.

[1] Armstrong, *Proc. Roy. Soc.*, **76**, (*B*) 606 (1905), studied the addition of various acids to the castor bean lipase and the dependence of the activity upon the acid added. This question was also studied by Connstein, Hoyer and Wartenberg, *Ber.*, **35**, 3988 (1902), Hoyer, *Z. physiol. Chem.*, **50**, 414 (1904) and others.

[2] Experiment (1) is omitted on account of the initial higher acidity.

With ethyl butyrate, somewhat different results were found (experiments 5–8 are included in this discussion). At 38°, for 20–23 hours, and for the amounts of alkali added of 0, 0.2, 0.27, 0.45 cc. (5, 25, 27, 29) the corresponding actions were 1.00, 0.85, 0.70, 0.50 cc. alkali, respectively. For 44–50 hours (6, 26, 28, 30) the actions were 1.60, 0.60,[1] 0.85, 0.55 cc., respectively. The addition of alkali evidently exerted a marked influence here. With none present, the ratio between the amounts of action for the two-time intervals was about the same as with the experiments with methyl acetate, obeying Schütz's rule, but the actions decreased and the differences between the amounts of action for the two-time intervals also decreased as the amount of alkali added initially was increased.

With olive oil, the amount of action for the shorter time interval was very small in every experiment (10, 32, 34, 36, 38), in no case exceeding 0.2 cc. alkali. The initial presence of alkali produced no apparent effect on the amount of hydrolysis. For the longer time intervals and 0.2 gram lipase preparation, the action varied from 0.05 cc. alkali at 20° (33), to 0.25–0.80 cc. alkali at 38° (35, 37, 39), with no apparent regularity as regards the amount of alkali added initially. A number of experiments were carried out in which the olive oil was used as an emulsion formed by adding its solution in a small quantity of acetone to the desired amount of water. The lipase preparation and either acid or alkali as desired, were then added and the further manipulations performed as in the other experiments. The results obtained showed no more satisfactory regularities than those which have been quoted with unemulsified olive oil, and are therefore not given in detail. In all these experiments with olive oil, whether emulsified or not, after adding the desired constituents, the mixtures were thoroughly agitated for a few seconds and then allowed to remain at the temperature in question until titrated (at the room temperature).

Experiment 31 is shown for the sake of comparison. The ester-solvent correction is large because of the hydrolysis of the ester by the hydrochloric acid. This correction is of doubtful accuracy, as the acid added to a lipase preparation, such as used here may well have been taken up by some basic body, resulting in a much smaller acidity than was used in the correction experiment.

In the experiments in which alkali was added initially, the weight of lipase preparation used required 0.2 cc. 0.1 *N* sodium hydroxide solution for neutrality to be attained. The first action of any added alkali doubtless would be to neutralize this small amount of free acid. Any further addition of alkali would, in the course of a short time, be neutralized by the acid formed by the saponification of the ester added

[1] This is the value from (26) and as stated before, is probably low (0.4–0.6 cc.).

(with the simple esters at any rate) or by the acid produced in some way from the lipase preparation (as made evident by the lipase-solvent experiments). Before this takes place, however, certain changes may be effected in the medium by the free alkali, such as the formation of an emulsion, temporary or permanent, or in the lipase preparation itself, and these unknown changes may influence the hydrolyzing power of the lipase. In the experiments with methyl acetate, alkali appears to exert no lasting influence on the amount of hydrolysis or acid formation from lipase and ester, as very nearly the same results were obtained whether alkali was added or not. This may have been due to the greater solubility of this ester as compared with the other esters, or to the rapid neutralization of the small excess of alkali added by the hydrolysis of the ester, removing the alkali before any permanent change was caused in the solution. With ethyl butyrate the results show a different action and evidently there is some direct influence of the alkali on the reaction. With olive oil the results do not point to any definit conclusion.

The small acidity of the lipase preparation represents the total free acids present and since these are doubtless organic acids,[1] the degree of ionization and therefore the H^+ ion concentration must be very small. In order to test the possible action due to the H^+ ions in a somewhat different way, some experiments were carried out in ether and in acetone solutions containing small amounts of water in which the small H^+ ion concentration would be expected to be very much decreased.

In ether saturated with water as solvent, (40) shows the action with methyl acetate to be about one-third as great as in aqueous solution (3). The action at 20° appeared to be approximately proportional to the weight of lipase preparation used (40–41). In (42), the solutions were boiled, using reflux condensers, and an amount of action was obtained in $6^1/_4$ hours at this temperature, 35–40°, about equal to the action which would have been obtained with the same weight of lipase preparation at 20° in 48 hours.. In acetone containing small amounts of water (43–44) there was less action than in ether, but at 20°, with 0.5 gram of lipase preparation there was still considerable action in 42 hours. In (45) the solutions were boiled (reflux condensers) and in spite of the high temperatures of the boiling acetone solutions, a fair amount of action was shown.

With ethyl butyrate and ether saturated with water, or acetone containing water as solvent, action was obtained only when 1.0 gram of lipase preparation was used (49–51). With olive oil and these solvents (52–53), the action was only slight under the same conditions.

The extraction of the active constituent of the castor bean by water and by ethyl acetate was also studied.

[1] Mainly lactic acid according to Hoyer, *Loc. cit.*

Three grams of the lipase preparation were ground with 20 cc. of water in a mortar and filtered through a thin asbestos mat. The filtrate was yellow in color and clear, but on standing became opalescent. Portions of 2.5 cc. diluted with 25 cc. water and treated with methyl acetate for 18 hours at 38° gave the following results:

2.5 cc. extract 1 cc. MeAc 25 cc. water required 1.3 cc. 0.1 *N* NaOH.
2.5 cc. extract ..... 25 cc. water required 0.3 cc. 0.1 *N* NaOH.
..... 1 cc. MeAc 25 cc. water required 0.4 cc. 0.1 *N* NaOH.

The action was therefore found to be 0.6 cc. 0.1 *N* sodium hydroxide solution. Compared with experiments 1 and 2 in Table I, it is seen that the action is much less, especially when it is considered that a larger amount of lipase preparation was used. The same results was found by Taylor,[1] who stated that some lipase is contained in a solution after filtration through paper, but much less than in the original suspension.

The lipase preparation after the extraction with ether in the Soxhlet extractor was extracted for three days in the same apparatus with ethyl acetate. The solution after the extraction was yellow in color and contained a flocculent white substance in suspension. The ethyl acetate was evaporated under diminished pressure below 40° and the residue which was taken up with water formed a solution containing some white matter and a small amount of acid. This solution was divided into two parts (*a*) and (*b*) and tested with methyl acetate for 22 hours at 38° with the following results:

(*a*) 12.5 cc. water 2 cc. MeAc required 2.5 cc. 0.1 *N* NaOH.
(*b*) 12.5 cc. water ..... required 1.3 cc. 0.1 *N* NaOH.
.. 15.0 cc. water 2 cc. MeAc required 0.4 cc. 0.1 *N* NaOH.

An acid formation equal to 0.8 cc. 0.1 *N* sodium hydroxide solution was found, showing that some of the active constituent had been extracted by the ethyl acetate. The residue from the extraction showed the same activity as unextracted lipase preparation, indicating that only a small part of the active substance had been removed.

The behavior of an aqueous suspension of lipase preparation toward an electric current was also studied. One gram of the lipase preparation was mixed thoroughly with 60 cc. water and placed in a U-tube of 1 cm. bore. The house current was passed through a 16 candle power carbon filament lamp and then through the solution in the U-tube, connection being made by means of platinum electrodes dipping just below the surfaces of the liquid in the two arms of the tube. It was evident that 0.5 ampere was not passing as the lamp did not glow at all, but that a small current was flowing was shown by the small but regular stream of gas evolved at the cathode. The current was allowed to flow for two weeks. After two days the solid matter had settled in the lower curved part of

[1] *Loc. cit.*

the tube except for a small amount which adhered to the electrodes or floated at the surface. Only occasional bubbles of gas were evolved at the anode. After two weeks, the solutions were removed separately from the two arms of the U-tube by means of pipets. They were slightly cloudy on account of the small amount of solid which had not settled to the bottom. Two portions of 10 cc. each were taken from each electrode solution, diluted to 35 cc. with water, 1 cc. methyl acetate added to one portion of each, allowed to stand for 47 hours at 38° and then titrated with 0.1 *N* sodium hydroxide solution. The results follow:

	Cathode solutions.		Anode solutions.	
MeAc.....	1 cc.	...	1 cc.	...
cc. 0.1 *N* NaOH required..	18.60	18.70	43.65	33.80

The cathode solution showed no hydrolytic power toward methyl acetate, although considerable acid was present there. On the other hand, the anode solution showed a still greater quantity of acid and also a large hydrolytic action. The absence of any but a minute gas evolution at the anode may therefore be explained as being due to some oxidation taking place there, and while some of the hydrolysis may have been produced by the large amount of acid formed, it seems very probable that the (negative) complex, produced or developed in part at any rate by oxidation, showed a marked hydrolytic or activating power.

Summary.

A comparative study was made of the hydrolysis of methyl acetate, ethyl butyrate, and olive oil caused by the castor bean lipase. The effect of adding small amounts of alkali at the beginning of the action was studied. No appreciable influence on the subsequent hydrolysis was found with methyl acetate, but with ethyl butyrate the action was different, depending upon the amounts of alkali added. Corrections were introduced in every case for the acid formed from ester-solvent and lipase-solvent.

In ether saturated with water, and acetone containing a small amount of water as solvents, methyl acetate was hydrolyzed to a considerable extent with lipase both at the ordinary and at more elevated temperatures.

Small amounts of an active constituent were extracted from the lipase preparation by water and by ethyl acetate.

By the electrolysis in water of the lipase preparation, a substance was produced in the anode solution, probably by oxidation, showing marked hydrolytic action.

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