[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF MONTANA]

THE ENZYME HYDROLYSIS OF BENZYL SUCCINATE

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Introduction

The contention by Macht[1] that the antispasmodic action of the alkaloids of the papaverine group is due to the benzyl nucleus led him to prepare and study some simple organic esters of benzyl alcohol. The fact that these gave evidence of a similar action led Shonle and Row[2] and Volwiler and Vliet[3] to prepare and investigate still other benzyl esters. Bye[4] has made a special clinical study of dibenzyl succinate and reports it superior to benzyl benzoate, due to "ease of administration, safety of retention, freedom from nausea and after intestinal disturbances and greater benzyl strength."

Whether or not the action of these esters is due to benzyl alcohol set free or to the intact benzyl ester molecule is still in question. This can best be settled by a study of the hydrolysis of these esters and comparing the data thus found with the corresponding pharmacological and clinical tests. Accordingly, Shonle and Row[2] studied the hydrolysis of benzyl stearate, palmatate and benzoate by the lipase of the pancreas. Volwiler and Vliet[3] studied the rates of hydrolysis of a number of benzyl esters, including dibenzyl succinate in dil. alcoholic potassium hydroxide solution.

However, the work of Christman and Lewis[5] raises the question whether the hydrolysis of dibenzyl succinate in the intestinal tract by the lipase of the pancreas would not be different from that indicated by the alcoholic potassium hydroxide hydrolysis. These investigators found that the lipase of hog liver carried the hydrolysis of diethyl succinate and malonate to an equilibrium which corresponded to the removal of one ethyl group and that the mono-ethyl ester was unattacked.

In order to determine whether or not pancreatic lipase would show a similar selective action on di- and monobenzylsuccinates the following study has been made.

Experimental

The Preparation of the Esters.—Dibenzyl succinate was prepared by heating sodium succinate with a slight excess of benzyl chloride in an oil-bath at 130–140° for 28 hours. Upon cooling, the mixture was washed with water and the excess of benzyl

[1] Macht, *J. Pharmacol.*, **9**, 287 (1917); **11**, 263 (1918).

[2] Shonle and Row, THIS JOURNAL, **43**, 361 (1921).

[3] Volwiler and Vliet, *ibid.*, **43**, 1672 (1921).

[4] Bye, *J. Ind. Eng. Chem.*, **13**, 217 (1921).

[5] Christman and Lewis, *J. Biol. Chem.*, **47**, 495 (1921).

chloride was removed by steam distillation. The solution was allowed to cool and the benzyl succinate was filtered off and dried. It was purified by recrystallization from petroleum ether. The product melted at 51–52°; b. p. 245° at 15 mm.; yield, 35%.

Monobenzyl succinate was prepared by heating succinic anhydride with a slight excess of benzyl alcohol under a reflux condenser for 4 hours. The product was purified by drying on a porous plate and subsequent recrystallization from benzene; yield, 30%. The portion insoluble in benzene was identified as succinic acid. The monobenzyl succinate melted at 55–56°. Duplicate 1-g. portions titrated against a 0.0611 *N* sodium hydroxide solution required 78.20 and 78.30 cc., respectively. The theoretical amount required is 78.25 cc.

The Hydrolysis of the Esters by Lipase.—The practical insolubility in water of these esters made it necessary to study their hydrolysis in emulsion. The method used was similar in principle to that used by Shonle and Row,[2] but employed more dilute solutions. A 5% acacia emulsion was made of the ester and the aqueous extract of fresh hog pancreas in such a manner that each 50 g. of the emulsion contained 1.00 g. of the ester, the aqueous extract of 1 g. of the pancreas and 1 cc. of fresh bile. This emulsion was incubated in 50g. duplicate samples for the desired periods of time. During the incubation the samples were frequently agitated. At the end of the incubation period the samples were removed and immersed in boiling water for 10 minutes. They were then cooled and 70 cc. of a neutral mixture of 5 parts of alcohol and 1 part of ether was added. They were titrated with 0.0611 *N* sodium hydroxide solution using phenolphthalein as an indicator. The end-point was taken as that point at which the pink color persisted for 30 seconds. In each case a blank was made up in the same manner, without the ester. Thus, the correction was made for the hydrolysis of the fat contained in the pancreatic extract. The activity of the extract was checked by samples in which olive oil was substituted for the ester.

The results are stated as the average of duplicate samples in terms of cc. of 0.0611 *N* sodium hydroxide solution. For dibenzyl succinate the following results were obtained.

Time, hrs.....	.5	1	1.5	3	5	7	20
Alkali, cc.....	6.75	7.27	7.98	9.65	9.35	8.60	6.75

The point marked by 9.65 cc. corresponds to 17.67% hydrolysis if the monobenzyl ester is formed or 8.88% hydrolysis if succinic acid is formed.

In order to identify the hydrolysis product, an emulsion containing 10 g. of dibenzyl succinate was incubated for 5 hours. To the 500 g. of emulsion was added 500 cc. of water and 2 liters of 95% alcohol. The precipitated acacia was filtered off and the filtrate evaporated until all the alcohol was removed. That portion of the dibenzyl succinate which had dissolved in the water-alcohol mixture crystallized from the remaining water on cooling. It was filtered off and the filtrate evaporated to dry-

ness. The residue was extracted with hot absolute alcohol and the alcohol solution decolorized with boneblack. The bile acids were precipitated by the addition of an equal volume of ether. A separate test made on succinic acid showed that the amount which might possibly be present here would be soluble in such an alcohol-ether mixture. The filtrate from the bile acids was evaporated to dryness. The slight residue remaining was tested for succinic acid with negative results.

In the study of monobenzyl succinate a glycerol extract of the pancreas was used with the following results.

Time, hrs	.5	1	3	5	20
Alkali with ester present, cc.	86.8	87.0	87.5	87.2	86.9
Alkali of blank, cc.	10.2	10.5	11.1	10.8	10.4
Net alkali, cc.	76.6	76.5	76.4	76.4	76.5

A duplicate sample containing 1 cc. of olive oil in place of 1 g. of ester required 17.4 cc. of alkali after a 5-hour incubation; this leaves no doubt as to the activity of the enzyme preparation. The 1 g. of mono-ester would require 78.25 cc. of this alkali. Christman and Lewis[5] have previously shown that neutralization of such acid esters has little if any effect upon their hydrolysis by the pancreatic enzymes. The results would seem to indicate clearly that there was no splitting of monobenzyl succinate.

Summary

1. The lipase of the pancreas will hydrolyze dibenzyl succinate only to monobenzyl succinate.

2. Monobenzyl succinate is not hydrolyzed by this enzyme.

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THE PREPARATION OF FRUCTOSE[1]

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The methods described in the literature for preparing pure crystalline *d*-fructose (levulose) have not always proved satisfactory when used on a small scale to obtain this sugar for chemical and bacteriological research. Experiments in this laboratory have indicated that the causes contributing to failure in preparing levulose may be fairly assigned to the use either of impracticable methods or of methods which have been described in insufficient detail. As very uniform success has been experienced in the fractional crystallization of glucose and fructose, an attempt will be made

[1] Paper read at the 62nd meeting of the American Chemical Society, September 6-10, 1921.