The Crystallization of Liver Esterase

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Many investigators have attempted to prepare and purify liver esterase [1–7]. Baker and King [8] developed a procedure by which the esterase activity per milligram of total solid was increased twelvefold.

In the course of attempting to further purify liver arginase tests were systematically conducted for the presence of several enzymes. It was found that certain fractions obtained were extremely rich in esterase activity. The crystallization of the enzyme from these fractions was finally attained.

Method of assay. Horse liver was used as the source for the enzyme. The liver was obtained from the slaughter house, ground and treated three times with several volumes (4—5) of acetone and then it was spread on filter paper and left to dry at room temperature. The dry material was stored at 3° C. No loss of esterase activity was noticed on storage at this temperature for 3 months.

Ethyl butyrate was used as substrate for the assay. The procedure consisted of mixing 1.75 g of ethyl butyrate in 100 ml Erlenmeyer flask with 50 ml of H_2O. The mixture was incubated at 40° C for 5 minutes and then 1 ml of liver powder suspension, extract, or dilute solution of crystalline enzyme was added. A control determination using the boiled extract (5 minutes) was always made. Another control (0 time incubation) was also made at the beginning of each experiment to determine the initial amount of alkali necessary for neutralization of the mixture. After 30 minutes incubation the solutions were titrated with 0.1 *N* alkali using phenolphthalein as an indicator.

Preparation of crystalline liver esterase. 300 g of the acetone dried liver is extracted with 3 l of 2.5 % solution of sodium acetate. The extraction is carried out at room temperature for two hours with frequent shakings. The pH is adjusted to 7.2 and the mixture is held at 55° C for 5 minutes in a water bath. It is cooled rapidly under running cold water and then centrifuged. The supernatent is stored at 3° C for several hours and then treated with 0.75 volumes of cold acetone (3—5). The mixture is left in the cold room over night. The pink flocculent precipitate that has settled is separated by centrifugation. To the clear supernatent is added 0.45 volumes of cold acetone. A light cream colored quite flocculent precipitate is formed. This is left at 3° C for 10 hours after which it is centrifuged and the precipitate is taken up in 1/10 the original volume of water. The pH is again adjusted to 7.2 and the mixture is centrifuged, discarding the precipitate. The supernatent fluid is heated at 65° C in a water bath for 2 minutes and cooled rapidly. A clear extract is obtained by centrifugation. This is then dialyzed at 3° C against a cold mixture of acetone and water (55 % acetone) for 15 hours. The recovered precipitate is suspended in water (1/30 of the original volume) and then centrifuged discarding the insoluble precipitate. Solid ammonium sulfate is added to the clear extract to render it 60 % saturated. The precipitate formed at room temperature is removed after 10 hours and discarded. It consists of amorphous proteins and the structures claimed by Bach [5] to be crystalline arginase. The partially turbid extract is left standing and in a few hours crystals of esterase begin to appear. This process can be hastened by making the extract 65 % saturated with respect to ammonium sulfate.

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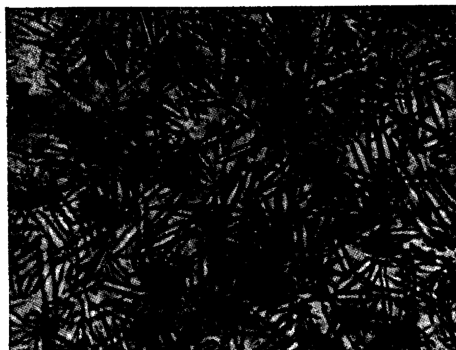

Figur 1. Crystalline horse liver esterase (× 450).

Recrystallization. The crystalline precipitate is separated and taken up in 1/40 of the original volume of extract. Enough ammonium sulfate is added to make it 60—65 % saturated, and the mixture is left standing at room temperature. Crystallization starts slowly but a good crop of crystals is obtained in 24 hours. The crystals (Fig. 1) have needle shape. 1 ml of crystalline enzyme containing 0.1 mg nitrogen when allowed to act on 1.75 g of ethyl butyrate will produce anough acid to neutralize 35.0 ml of 0.01 N sodium hydroxide in one half hour.

Work is in progress to study the purity of the preparation electrophoretically and by solubility methods. Investigations on the kinetics, specificity and activation inhibition reactions are also being carried out and will be communicated in more detail in a later publication.

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Sulphanilylderivatives of Heterocyclic Amines. XI.* Derivatives of Quinoxaline

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In connection with researches on the bacteriostatic effect of isomeric N^1-heterocyclic derivatives of sulphanilamide [1] it was desired to prepare the three isomeric sulphaquinoxalines. It was attempted to prepare 2-amino-quinoxaline from *o*-phenylenediamine plus glyoxylic nitrile or dichloroacetonitrile or from quinoxaline and sodium amide, but with negative results. While these experiments were still in progress, Weijlard, Tishler and Erickson published a paper [2] in which the synthesis of 2-sulphaquinoxaline and 2-aminoquinoxaline, starting from alloxazine, was announced. 2-Sulphaquinoxaline was found to be as effective as sulphadiazine in experimental pneumococcus infections in mice.

The two remaining isomers, 5- and 6-sulphaquinoxaline, have now been prepared by treating the appropriate aminoquinoxalines with acetylsulphanilyl chloride in the presence of pyridine and hydrolyzing the resulting acetyl derivatives. The two aminoquinoxalines required were prepared from 1,2,4- and 1,2,3-triaminobenzene and glyoxal. The 6-aminoquinoxaline

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