T-IV-3-2	Lys
T-IV-7-2	Leu, Arg
T-V-1-1	Arg
T-V-1-2	Val (Asp, Ser, Glu, Ala, Lys)
T-V-2-2	Phe (Glu, Arg)
T-V-3-1-2	Val (Asp, Thr, Glu, GLy, Ala, Phe, His, Lys)
T-V-3-2	Val (Ser, Gly, Arg)
T-VI-3-2	Thr (Asp, Glu, His, Lys)
T-VI-5-1	Phe (Asp, Glu, Gly, His, Arg)

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SPECIFICITY OF THE TRIACETINASE OF COTTON SEEDS

Sh. S. Azimova and P. Kh. Yuldashev

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The isolation of triacetinase – an enzyme which has been classified as a lipase – has been reported previously [1].

As Desnuelle and Sarda [2] have shown, the main difference between lipases and esterases is shown in their relationship to the physical state of the substrate – the lipases, unlike esterases, do not hydrolyze water-soluble substrates. This is shown best using the reaction with triacetin as example. This compound is readily soluble in water at low concentrations, and at higher concentrations it gives stable emulsions. In the case of lipases, hydrolysis takes place at an appreciable rate only when the concentration of triacetin is sufficiently high for the formation of micelles. In the case of esterases, hydrolysis begins at very low concentrations of triacetatin.

The results of our experiments are shown in Fig. 1. It is obvious from this graph that the triacetinase of cotton seeds is an esterase, K_m for this reaction being $1.6 \cdot 10^{-4}$.

When the triacetinase was incubated with diisopropyl phosphorofluoridate in a concentration of 10^{-3} M, the enzyme was 100% inactivated.

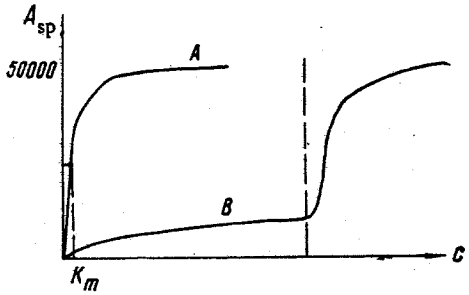

Fig. 1. Curves of the dependence of the rate of hydrolysis by triacetinase on the concentration of triacetin (A) and a typical curve of the hydrolysis of triacetin by lipases (B). The broken line denotes the limit of solubility of triacetin.

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Reactivities were measured on a "Radiometer" titrator (Denmark) without the use of buffer solutions. As the unit of activity we took the number of micromoles of acid liberated in unit time.

Thus, it has been shown that the triacetinase of cotton seeds is an esterase belonging to the class of serine proteinases.

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