COMMUNICATIONS TO THE EDITOR

CRYSTALLIZATION OF ALPHA-AMYLASE FROM A THERMOPHILIC BACTERIUM

Sir:

We have studied the thermal stability of highly purified α-amylase produced at 35 and 55° by two facultative thermophilic bacteria, *Bacillus coagulans* and *B. stearothermophilus*. It was demonstrated that the 55° preparations were more heat stable than the 35° preparations losing only 6 to 10% of their activity in one hour at 90° whereas the latter preparations showed a reduction in activity of 90 to 92% when tested under identical conditions.[1] It has not been possible, however, to state whether the thermal stability of the 55° preparations is an inherent function of the protein structure or whether it is due to protective effects of impurities still present in the purified systems. The solution to this problem had to await the isolation of the crystalline enzymes so that the influence of accompanying impurities could be avoided. The present communication describes the crystallization of alpha-amylase from a facultative thermophilic bacterium, *Bacillus coagulans* (43P-4). The medium for enzyme production and the method of enzyme assay was that described previously.[1] Incubation was at 55° for 24 hours. After the removal of cells by centrifugation at 20,000 r.p.m. in a Sharples centrifuge the supernatant liquid, *ca.* 9 l., was concentrated to one-third the original volume by evaporation under reduced pressure.

The enzyme was isolated by a sodium sulfate–ammonium sulfate fractionation, followed by dialysis against 0.01 *M* calcium acetate. Further purification was accomplished by two acetone fractionations at −10°, followed by an ammonium sulfate fractionation (0.65 saturation). The crystalline enzyme was prepared as follows: the 0.65 ammonium sulfate fraction was dissolved in 0.2 *M* calcium acetate and adjusted to *p*H 6.0. Solid ammonium sulfate was slowly added to 0.25 saturation and allowed to stand at 30° for 3 hours. To the slightly turbid solution was added solid ammonium sulfate to a final saturation of 0.33. After standing overnight at 30°, the enzyme crystallized in the form of long, white, shiny needles. Recrystallization was accomplished by repeating the 0.25 and 0.33 ammonium sulfate fractionations. The final crystalline product (5 × recrystallized) represented a 1,000-fold increase in enzyme activity over the starting material. Solubility and electrophoretic measurements have shown that the crystalline enzyme is homogeneous in character.

Amylase elaborated at 35° by this organism has also been obtained in crystalline form using the methods described herein. A comparison of the heat stability of the two crystalline preparations revealed that the 55° preparation retained 88 to 90% of its activity after 60 minutes at 90° whereas the 35° preparation retained only 6 to 10% of its activity under identical conditions, thus confirming our earlier findings with highly purified but noncrystalline preparations.[1] To the author's knowledge this is the first report of the isolation of a crystalline enzyme from a thermophilic bacterium. A detailed study of the properties of the two crystalline preparations is now in progress and will be reported on later.

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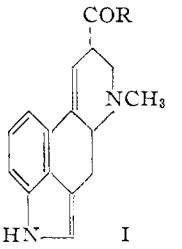
(3) This investigation was initiated in the laboratory of Dr. O. B. Williams, Department of Bacteriology, University of Texas, Austin, while the author was a Predoctoral Research Fellow of the National Institutes of Health. The author wishes to thank Dr. O. B. Williams and Dr. H. A. Barker for the many courtesies extended and helpful suggestions made during his stay in their laboratories.

THE TOTAL SYNTHESIS OF LYSERGIC ACID AND ERGONOVINE

Sir:

The striking physiological effects attributable to ergot have been known since pre-Christian times, and were familiar to mediaeval Europe, where the ingestion of grain infected by the fungus *Claviceps purpurea* not infrequently caused outbreaks of the dread malady known as St. Anthony's Fire. More recently, the active principles have been shown to be amides of lysergic acid (I, R = —OH), of which the simplest is ergonovine (I, R = —NH-$CH(CH_3)CH_2OH$), whose oxytocic effect has led to its widespread use in obstetrical medicine. We now wish to record the first total synthesis of lysergic acid.



I

The reaction of N-benzoyl-3-(β-carboxyethyl)-dihydroindole[1] with thionyl chloride, followed by aluminum chloride, gave 1-benzoyl-5-keto-1,2,2a,-3,4,5-hexahydrobenz[*cd*]indole (II, R = —CO-C_6H_5, R′ = H) (m.p. 148–150° (uncor.); calcd. for $C_{18}H_{15}NO_2$: C, 77.96; H, 5.45; N, 5.05. Found: C, 77.81; H, 5.29; N, 5.15). Bromination of the tricyclic ketone gave the 4-bromo derivative (II, R = —COC_6H_5, R′ = Br) (m.p. 181–182°; calcd. for $C_{18}H_{14}BrNO_2$: N, 3.94; Br, 22.44. Found: N, 3.94; Br, 22.14), which was converted by reaction with methylaminoacetone ethylene ketal to the ketal-ketone (II, R = —COC_6H_5; R′ =

(1) B. K. Blount and R. Robinson, *J. Chem. Soc.*, 3158 (1931).