# COMMUNICATIONS TO THE EDITOR

## CRYSTALLIZATION OF ALPHA-AMYLASE FROM A THERMOPHILIC BACTERIUM

*Sir:*

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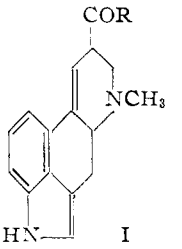
(3) This investigation was initiated in the laboratory of Dr. O. B. Williams, Department of Bacteriology, University of Texas, Austin, while the author was a Predoctoral Research Fellow of the National Institutes of Health. The author wishes to thank Dr. O. B. Williams and Dr. H. A. Barker for the many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

## THE TOTAL SYNTHESIS OF LYSERGIC ACID AND ERGONOVINE

*Sir:*

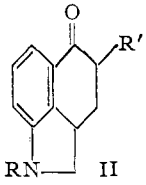
The striking physiological effects attributable to ergot have been known since pre-Christian times, and were familiar to mediaeval Europe, where the ingestion of grain infected by the fungus *Claviceps purpurea* not infrequently caused outbreaks of the dread malady known as St. Anthony's Fire. More recently, 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.



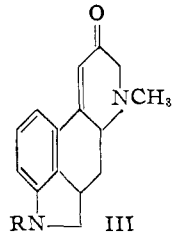

I

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).

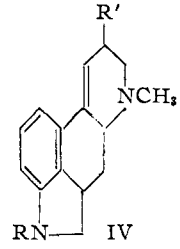


II



III

—$N(CH_3)CH_2C(CH_3)OCH_2CH_2O$) (m.p. 135–136°; calcd. for $C_{24}H_{26}N_2O_4$: C, 70.91; H, 6.45; N, 6.89. Found: C, 70.95; H, 6.49; N, 6.95). Acid hydrolysis of the latter yielded the diketone (II, R = H, R′ = —$N(CH_3)CH_2COCH_3$) (m.p. 109–110°; calcd. for $C_{15}H_{18}N_2O_2$: C, 69.74; H, 7.02; N, 10.85. Found: C, 70.00; H, 7.41; N, 10.91), which on treatment with sodium methoxide was converted to the tetracyclic ketone (III, R = H) (m.p. 155–157°; calcd. for $C_{15}H_{16}N_2O$: C, 74.97; H, 6.71; N, 11.66. Found: C, 75.08; H, 6.95; N, 11.78). Acetylation of the ketone afforded (III, R = —$COCH_3$) (m.p. 169–170°; calcd. for $C_{17}H_{18}N_2O_2$: C, 72.32; H, 6.43; N, 9.92. Found: C, 72.61; H, 6.53; N, 9.75), which on reduction with sodium borohydride gave the alcohol (IV,



IV

R = —$COCH_3$, R′ = OH) (m.p. 187–188°). The *hydrochloride* (m.p. 245–246° (*dec.*); calcd. for $C_{17}H_{21}N_2O_2Cl$: C, 63.64; H, 6.60; N, 8.73. Found: C, 63.47; H, 6.81; N, 8.96) of the latter, when treated with thionyl chloride in liquid sulfur dioxide, furnished an amorphous *chloride hydrochloride*, which was converted by sodium cyanide in liquid hydrogen cyanide to the nitrile (IV, R = —$COCH_3$; R′ = —CN) (m.p. 181–182°; calcd. for $C_{18}H_{19}N_3O$: C, 73.69; H, 6.53; N, 14.33. Found: C, 73.41; H, 6.53; N, 14.17). Methanolysis of the nitrile gave the ester (IV, R = H, R′ = —$COOCH_3$) (m.p. 160–161°; calcd. for $C_{17}H_{20}N_2O_2$: C, 71.80; H, 7.09; N, 9.85. Found: C, 71.86; H, 7.19; N, 10.05). Alkaline hydrolysis of the latter, followed by catalytic dehydrogenation in water using a deactivated Raney nickel catalyst[2] gave *dl*-lysergic acid (I, R = —OH) (m.p. 241–242° (*dec.*); calcd. for $C_{16}H_{16}N_2O_2$: C, 71.62; H, 6.01; N, 10.44. Found: C, 71.51; H, 6.10; N, 10.32). The synthetic *dl*-lysergic acid was converted to the corresponding ester by means of diazomethane and thence with hydrazine to *dl*-isolysergic acid hydrazide (I, R = —$NHNH_2$) (m.p. 224–227° (*dec.*); calcd. for $C_{16}H_{18}N_4O$: C, 68.06; H, 6.43; N, 19.85. Found: C, 68.00; H, 6.44; N, 19.76). Both the acid and hydrazide were identical with the corresponding samples derived from natural ergot alkaloids[3,4] in melting point, mixture melting point, ultraviolet spectrum, infrared spectrum, paper chromatographic behavior and X-ray diffraction pattern.

Since *dl*-isolysergic acid hydrazide (I, R = —$NHNH_2$) has already been resolved and reconverted to ergonovine (I, R = —$NHCH(CH_3)CH_2OH$),[5] the present work constitutes also a total synthesis of this ergot alkaloid.

(2) E. C. Kleiderer and E. C. Kornfeld, *J. Org. Chem.*, **13**, 455 (1948).

(3) S. Smith and G. M. Timmis, *J. Chem. Soc.*, 1440 (1936).

(4) A. Stoll and A. Hofmann, *Z. physiol. Chem.*, **250**, 7 (1937).

(5) A. Stoll and A. Hofmann, *Helv. Chim. Acta*, **26**, 922, 944 (1943).

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## FORMATION OF BICYCLO(4.1.0)HEPTANE DERIVATIVES FROM EUCARVONE

*Sir:*

Reaction of eucarvone (I) with selenium dioxide in ethanol yields 40% of a crystalline hydroxy derivative, m.p. 85–86°, $\lambda_{max}$ 229 mμ (log ε 4.06), $\nu_{max}$ 3610, 3408, 1659, 1641 cm.$^{-1}$ (*Anal.* Calcd. for $C_{10}H_{14}O_2$: C, 72.26; H, 8.49. Found: C, 72.00; H, 8.46; 1 double bond (Pd–C catalyst)) to which, on the basis of these data and the following evidence, we assign the *bicyclic* structure II. The hydroxy ketone II is transformed by oxidation with manganese dioxide into the diketone III, m.p. 93–94°, $\lambda_{max}$ 240 mμ (log ε 3.92), $\nu_{max}$ 1670, 1628 cm.$^{-1}$ (*Anal.* Calcd. for $C_{10}H_{12}O_2$: C, 73.14; H, 7.37. Found: C, 73.19; H, 7.25. 1 double bond (Pd–C catalyst)). Oxidation of the diketone III with neutral permanganate produces the known *cis*-carconic acid,[1,2] m.p. 175.5–176.5°, $\nu_{max}$ 3180, 1725, 1685 cm.$^{-1}$ (*Anal.* Calcd. for $C_7H_{10}O_4$: C, 53.16; H, 6.37. Found: C, 53.22; H, 6.57), monomethyl ester, m.p. 107–109°, which establishes the presence of the *gem*-dimethylcyclopropyl unit in II and III.

Eucarvone is oximated by butyl nitrite–sodium ethoxide–ethanol to give a 72% yield of the *bicyclic* oximino ketone IV, m.p. 153–154°, $\lambda_{max}$ 222,296 mμ (log ε 3.92, 4.00), $\nu_{max}$ 3585, 3275, 1648 cm.$^{-1}$ (*Anal.* Calcd. for $C_{10}H_{13}O_2N$: C, 67.00; H, 7.31; N, 7.82. Found: C, 67.12; H, 7.30; N, 7.78). The same oximino ketone could be prepared by addition of the sodio derivative of I in dioxane to cold, ethereal butyl nitrite. The oximino ketone IV was correlated with the diketone III by conversion to the same dioxime V, m.p. 185–186°, $\lambda_{max}$ 278 m (log 4.43), $\nu_{max}$ 3250, 3195, 1625(w) cm.$^{-1}$ (*Anal.* Calcd. for $C_{10}H_{14}O_2N_2$: C, 61.83; H, 7.27; N, 14.42. Found: C, 61.62; H, 7.00; N, 14.28).

In order to gain information regarding the possible courses for the formation of bicyclo[4.1.0]-heptane derivatives from eucarvone, we have studied the ethoxide-catalyzed hydrogen–deuterium

(1) W. H. Perkin and J. F. Thorpe, *J. Chem. Soc.*, **75**, 48 (1903).

(2) K. Hariharan, K. Menon and J. Simonsen, *ibid.*, 431 (1928).