wt., 363); TNM and Br_2 tests negative; no absorption at 205–300 mμ; no infrared hydroxyl or carbonyl bands; characteristic bands (CS_2) at 8.90, 9.22, 9.85, 10.22, 10.65, 11.53, and 12.54 μ; proton resonance peaks at 82 and 94 cps. in $CDCl_3$ (referred to benzene resonance peak; no other peaks in the range 0–160 cps.) indicating two hydrogens attached to different oxygen-linked carbons. The oxide is stable to hydrochloric acid in refluxing methanol–dioxane. That cyclization is not attended with rearrangement of the ring system was established as follows. When heated with *p*-toluenesulfonic acid in acetic anhydride the oxide afforded an oily unsaturated acetate which on hydrogenation and hydrolysis yielded B-norcoprostane-6α-ol, m.p. 102.5–103.5°, $[\alpha]_D$ −20° (C, 83.32; H, 12.30). Oxidation gave the 6-ketone, m.p. 98–98.5°, $[\alpha]_D$ +34°, λ^{CS_2} 5.77 μ (C, 83.59; H, 11.79). An identical ketone was obtained from B-norcoprostane-3,6-dione by partial reduction with sodium trimethoxyborohydride to an oil containing predominantly the 3-ol-6-one. Conversion to an ethyleneketal mixture, oxidation, and chromatography on alumina gave B-norcoprostane-3,6-dione 6-ethyleneketal, m.p. 120–121°; $[\alpha]_D$ +50°, λ^{CS_2} 5.83 μ (C, 77.76; H, 10.83). Wolff–Kishner reduction of the ketol and hydrolysis gave B-norcoprostane-6-one, identical with the above sample (m.p., $[\alpha]_D$, infrared).

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A CRYSTALLINE DECARBOXYLASE WITHOUT BIOTIN

Sir:

During an investigation of the mechanism of decarboxylation[1] we have crystallized the decarboxylase for acetoacetic acid from *Clostridium acetobutylicum*. The microörganism (American Type Culture Collection No. 862) was grown and harvested and the crude enzyme prepared by a modification of the procedure developed by Davies[2] and Seeley.[3] The partially purified enzyme was comparable in activity to the best samples obtained by Davies. Approximately 25 mg. of this preparation in 3 ml. of solution was dialyzed against 0.05 *M* phosphate buffer at *p*H 5.9 and then placed on a column of diethylaminoethyl cellulose.[4] On gradient elution to 0.05 *M* phosphate (*p*H 5.9)–0.1 *M* ammonium sulfate, the enzyme was spread over several fractions; these were combined and precipitated with 60% saturated ammonium sulfate. The precipitate was suspended in 1 ml. of 0.05 *M* phosphate (*p*H 5.9) and, on standing at room temperature, microscopic hexagonal plates of the enzyme crystallized out. The enzyme can be recrystallized from a solution in 0.05 *M* phosphate (*p*H 5.9) by adding ammonium sulfate to about 50% saturation. The crystalline enzyme is 2 to 3 times as active as Davies' best preparation. In a Spinco model E ultracentrifuge, the protein proved monodisperse, and showed a sedimentation constant (extrapolated to zero concentration) of about 15. Its molecular weight is then presumably of the order of 300,000.

Samples of the enzyme were hydrolyzed with 6 *N* sulfuric acid or 4.5 *N* potassium hydroxide solution at 120° for two hours, and the hydrolysate assayed microbiologically[5] for biotin, using *Saccharomyces cerevisiae* (American Type Culture Collection No. 7754). Control experiments showed that the vitamin, as measured by this assay, was not extensively destroyed under the experimental conditions employed; the method gave results approximating those in the literature for biotin-containing tissues.[6] The assay with hydrolyzed enzyme gave results which did not differ significantly from "blanks." Assuming that the concentration of the enzyme could be determined from the absorption in the 260–280 mμ region and that the enzyme was hydrolyzed under the above conditions, the data show that the enzyme contains less than 0.01 molecule of biotin per 300,000 molecular weight units. This vitamin has been implicated, and is presumably present, in another beta ketoacid decarboxylase,[7] and in the carboxylating enzyme in fatty acid synthesis.[8] Biotin may prove essential for the fixation of carbon dioxide by certain enzymes, but it is not present in the pure crystalline decarboxylase for acetoacetic acid.

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A NEW AND GENERAL METHOD FOR THE ISOLATION OF ANTI-PROTEIN ANTIBODIES

Sir:

Although a few general methods for the isolation of pure antibodies (Ab) directed against protein antigens (Ag) have been described,[1] the need exists for a convenient, reproducible and gentle method capable of good yields. We have developed such a method with which we have so far isolated pure

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