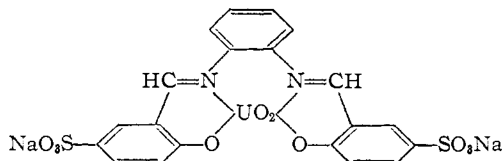
We wish to report here the synthesis of the complex, uranyl bis-(sulfosalicylal)-*o*-phenylenediamine, which was prepared in pure crystalline form as the sodium salt, and is being applied as a heavy-atom dye in the crystal-structure analysis of the protein bovine pancreatic ribonuclease. The complex is taken up readily by crystals of ribonuclease from a 2.5×10^{-4} *M* solution in 75 volume % 2-methyl-2,4-pentanediol. This complex is presumed to have the structure



The method of preparation involves simply the mixing in aqueous solution of sulfosalicylaldehyde (prepared according to Blau[3]), *o*-phenylenediamine, uranyl acetate and sodium hydroxide in the molar ratio 2:1:1:2, and crystallization from aqueous isopropyl alcohol. The analytical results for uranium are in accord with the proposed composition (Calcd. for $C_{20}H_{12}N_2S_2O_{10}Na_2U$: U, 30.3. Found: U, 31.5).[4] In the absence of further analytical data or degradative studies, the proposed structure cannot be proved, but is rendered highly probable by analogy with known complexes and consideration of steric requirements. The assigned octahedral coördination of uranium(VI) is not the most usual for this element, but has been observed previously in crystalline compounds, *e.g.*, $BaUO_4$.[5]

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(3) F. Blau, *Monatsh.*, **18**, 123 (1897).
(4) Virginia Hong, Master's Thesis, Polytechnic Institute of Brooklyn, June, 1957.
(5) S. Samson and L. G. Sillen, *Ark. f. Kem. Min. och Geol.*, **25A**, No. 21, 1 (1947).

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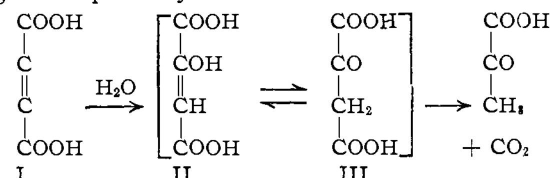
ENZYMATIC UTILIZATION OF AN ACETYLENIC COMPOUND

Sir:

A soluble enzyme system catalyzing the conversion of acetylenedicarboxylic acid (I) to equimolar quantities of pyruvic acid and carbon dioxide has been partially purified from sonic extracts of a species of *Pseudomonas* isolated from soil. The enzyme system has been purified 50-fold by protamine treatment, ammonium sulfate and acetone precipitation and by adsorption and elution from calcium phosphate gel. The most active preparations catalyze the formation of 55 μmoles of pyruvate[1] per minute per mg. protein at 25° and *p*H 7.3.

After incubation of the enzyme with I, the 2,4-dinitrophenylhydrazone of pyruvic acid was isolated (m.p. 215°). The reaction is experimentally irreversible as demonstrated by the lack of incorporation of C^{14} into acid-stable linkages when $C^{14}O_2$ is incubated with the enzyme and either I or pyruvate. In a preliminary report Eimhjellen[2] has noted that oxalacetate (III) is formed by resting cells, and pyruvate by extracts, of an enterobacterium exposed to ADA. This finding would suggest the pathway shown



However, results with the purified enzyme from *Pseudomonas* clearly eliminate *free* oxalacetate as an

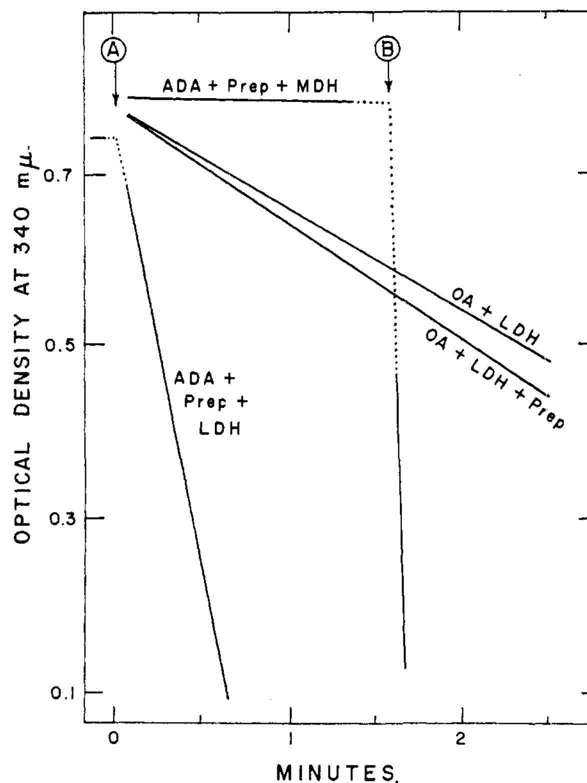


Fig. 1.—Tracing obtained from a Cary recording spectrophotometer of the rate of reduced pyridinenucleotide (DPNH) oxidation. All incubation mixtures contain 50 μmoles of phosphate at pH 7.3 and 0.1 μmoles of DPNH per ml. When applicable the following were added to a total volume of 1.0 ml.: ADA, acetylene dicarboxylate, 10 μmoles, Prep, *Pseudomonas* enzyme, 2 μg.; OA, oxalacetate freshly prepared, 2 μmoles; LDH and MDH, an excess of lactic dehydrogenase and malic dehydrogenase, respectively. Arrows A and B denote the addition of substrate and oxalacetate, respectively.

(1) Assayed by the method of T. E. Friedemann and G. E. Haugen, *J. Biol. Chem.*, **147**, 415 (1943).
(2) K. E. Eimhjellen, *Biochem. J.*, **64**, 4 p. (1956).

intermediate on the following grounds: (1) oxalacetate as assayed by the method of Neish[3] does not accumulate. (2) The oxalacetic decarboxylating activity (both spontaneous and enzymatic) of these preparations is too low to account for the rapid rate of pyruvate formation from I (Fig. 1). (3) Addition of malic dehydrogenase to the incubation mixture (Fig. 1) indicates the absence of free oxalacetate. These observations do not exclude an enzyme-bound form of oxalacetate from participating in the reaction as shown.

The decarboxylation of I to form propiolic acid (CH⋮CCOOH) has been ruled out as the latter compound is not active in this system. The possibility of an enzyme-propiolate complex seems remote as the formation of such a bound form might be expected to be reversible and result in the incorporation of radioactivity when $C^{14}O_2$ was present. Further purification and studies on the mechanism of the reaction are in progress.

(3) W. J. P. Neish, In D. Glick, "Methods of Biochemical Analysis," Vol. 5, Interscience Publishers, Inc., New York, N. Y., 1957, p. 168.

(4) Fellow of The Jane Coffin Childs Memorial Fund for Medical Research. This investigation has been aided by a grant from The Jane Coffin Childs Memorial Fund for Medical Research.

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ROLE OF BIOTIN IN CARBAMYLATION REACTIONS

Sir:

Biotin deficiency in *Streptococcus lactis* 8039 is associated with a loss of ability to convert ornithine and carbamyl phosphate to citrulline.[1] Carbamyl phosphate is involved in the conversion of aspartate to N-carbamylaspartate,[2] a precursor of pyrimidines, and a study of biotin sulfone-inhibition of the growth of *Lactobacillus arabinosus* 17–5 suggested a possible role of biotin in pyrimidine biosynthesis.[3]

In this investigation, a comparison of cell-free extracts of normal and biotin-deficient *L. arabinosus* revealed that the latter have a greatly diminished ability to carbamylate aspartate as well as ornithine[4] as indicated in Table I. Specificity of this effect is indicated by the lack of an effect of biotin deficiency upon α-ketoglutarate-aspartate transaminase activity. Biotin or heat-inactivated extracts of normal cells do not restore activity to biotin-deficient cell extracts. Normal activity can be restored to biotin-deficient cells in a biotin supplemented growth medium in a few hours.

Protein synthesis in the presence of biotin is essential for the formation of the ornithine-citrulline enzyme in biotin deficient cells of *S. lactis*.[5] Purification of this enzyme from extracts of *S. lactis* by ammonium sulfate fractionation (60 to 75% saturation) at *p*H 7, heat treatment (65° for 5 minutes), ammonium sulfate fractionation (50 to 75% saturation) at *p*H 8.5; and chromatography on diethylaminoethylcellulose gave a preparation with an activity of 73,000 (μmoles citrulline produced/mg. protein/hr.). Assayed with *Saccharomyces cerevisiae* after hydrolysis with 3.6 *N* sulfuric acid, the purified preparation and the original cell extract (activity, 960) gave 0.1 and 3 mμ g., respectively, of biotin/mg. protein. Unless biotin is present in the enzyme in a form which does not yield an active form of biotin upon acid hydrolysis, biotin apparently is not a component of the enzyme but exerts its effect during enzyme synthesis presumably in the formation of groups necessary for the transfer of a carbamyl group.

TABLE I

EFFECT OF BIOTIN-DEFICIENCY ON ENZYME ACTIVITY

Enzyme system	Activity of cell-free extracts,[a] μmoles of product formed/mg. protein/hr. Control	Biotin-deficient
Ornithine carbamylation[b]	144	16
Aspartate carbamylation[c]	18	1.3
α-Ketoglutarate-aspartate[d] transaminase	2.2	2.6

[a] From *L. arabinosus* grown in amino acid medium[1] containing no arginine or uracil and 20 or 0.3 mμg./ml. of biotin, respectively. [b] Extract incubated with carbamyl phosphate, 20 μmoles; tris-(hydroxymethyl)-aminomethane buffer, 10 μmoles; ornithine, 10 μmoles in 1 ml. at *p*H 8.3 for 30 minutes at 35°. Cirtulline determined colorimetrically.[6] [c] As in *b* with aspartate in lieu of ornithine; *p*H 7.5; incubated at 25°; passed through a Dowex-50 column before carbamylaspartate determined.[7] [d] Determined as previously described.[8]

(1) J. M. Estes, J. M. Ravel and W. Shive, THIS JOURNAL, **78**, 6410 (1956).

(2) M. E. Jones, L. Spector and F. Lipmann, *ibid.*, **77**, 819 (1955).

(3) J. M. Ravel and W. Shive, *Arch. Biochem. Biophys.*, **54**, 314 (1955).

(4) Previously reported inability of *L. arabinosus* to effect this reaction resulted from arginine inhibition of active enzyme formation; in contrast, *S. lactis* requires arginine for optimal enzyme synthesis.

(5) R. F. Sund, J. M. Ravel and W. Shive, *J. Biol. Chem.*, in press.

(6) R. M. Archibald, *J. Biol. Chem.*, **156**, 121 (1944).

(7) S. B. Koritz and P. P. Cohen, *ibid.*, **209**, 145 (1954).

(8) N. E. Tonhazy, N. G. White and W. W. Umbreit, *Arch. Biochem.*, **28**, 36 (1950).

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DETERMINATION OF PROTON AFFINITY AND BOND DISSOCIATION ENERGY BY ION IMPACT METHOD

Sir:

Until recently there were no experimental methods for the determination of the proton affinity of saturated molecules. A method for such a determination has been proposed by the authors[1] and it consists in investigating reactions between ions and molecules in the ionization chamber of a mass spectrometer. If, under the experimental conditions, a reaction can be observed, one may conclude that the reaction has no activation energy (to within an accuracy of 1–2 kcal./mole) and that it is either thermoneutral or exothermic. If a reaction is not observed one may conclude that it is endothermic. After a number of reactions have been investigated, the above criterion can be used to set up a series of inequalities and to determine thereby the limits within which either the proton

(1) V. L. Tal'rose and E. L. Frankevitch, *Doklady Akad. Nauk S.S.S.R.*, **111**, No. 2, 37 (1956).