CHEMICAL REACTIVITY OF MODELS RELATED TO A PROPOSED CO_2~BIOTIN-ENZYME COMPLEX.III.

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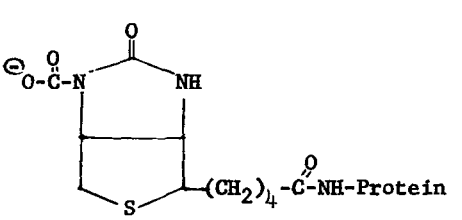
Since 1959 considerable progress has been made in the elucidation of the mechanism of carboxylation reactions which utilize a biotin-containing enzyme (Knappe, Ringelmann and Lynen, 1961; Waite and Wakil, 1963). The stoichiometry of many of these enzymatic carboxylation reactions may be outlined as (Kaziro et al., 1962.):

(1) ATP + $HCO_3^{\ominus}$ + Biotin-Enzyme $\overset{Mn^{+2}}{\rightleftharpoons}$ CO_2~Biotin-Enzyme +ADP + Pi

(2) CO_2~Biotin-Enzyme + acceptor $\rightleftharpoons$ Biotin-Enzyme + acceptor-$\overset{O}{\overset{\|}{C}}$-$O^{\ominus}$

where the acceptor is a nuleophilic reagent; frequently the nucleophilic reagent is an acyl Co A. Thus, if acetyl Co A is the nucleophilic reagent, the product of the enzymatic reaction is malonyl Co A.

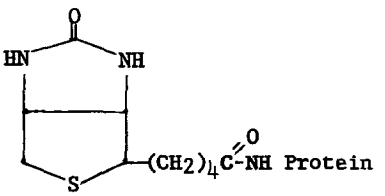
Recently, two concepts concerning the mechanism of reaction in related enzymatic carboxylation reactions have emerged: (1) in one of the concepts (Knappe, Ringelmann and Lynen, 1961), it is proposed that the reaction of $HCO_3^{\ominus}$ with a biotin-enzyme produces 1'-N-carboxybiotin-enzyme (I).



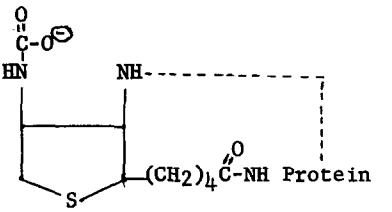
I

Attack on I by a nucleophilic reagent; e.g., an acyl Co A results in the formation of a carboxylated acyl Co A and regenerates the original biotin-enzyme. In this concept, then, the $\overset{\ominus}{O}\text{-}\overset{O}{\overset{\|}{C}}\text{-}$ of I represents the "active CO_2" in enzymatic syntheses of carboxylic acids.

(2) In the second concept (Waite and Wakil, 1963), it is proposed that either II or III represents the active form of the biotin-enzyme.



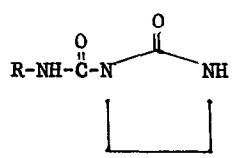
II



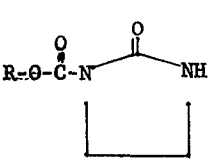
III

In the case of II, the carbonyl group of the imidazolidone ring is transferred to an acyl Co A, which then reacts with water to form the carboxylic acid. The resulting diaminobiotin could react with $HCO_3^{\ominus}$ to reform II. In the case of III, it is suggested that the acyl Co A attacks the carbonyl group of $\overset{O}{\overset{\|}{C}}\text{-}O^{\ominus}$ with the formation of the carboxylic acid and the diaminobiotin-enzyme. Diaminobiotin-enzyme can react with $HCO_3^{\ominus}$ to regenerate III.

In order to gain more information about the mechanism of these reactions, we undertook a study of certain model compounds related to the proposed CO_2~biotin-enzyme complex (Schaeffer and Bhargava, 1962). The model compounds which we studied initially were the N-arylcarbamyl-2-imidazolidones (IV) and the N-alkoxycarbonyl-2-imidazolidones (V).



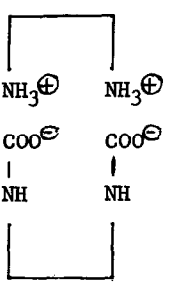
IV



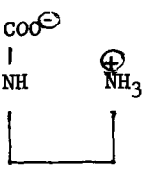
V

The model compounds IV and V are related to I, but are different in that the $C\text{-}O^{\ominus}$ of I is in the form of an amide or ester in IV and V. The amide and ester grouping was used because of the known instability of compounds with the $N\text{-}\overset{O}{\overset{\|}{C}}\text{-}O^{\ominus}$ as in I. The models IV and V did, in fact, transfer the carbonyl group to an attacking nucleophilic reagent, and several interesting ring openings were observed, but because of the low rate of reaction, it was felt that this type of structure could not represent the exact structures present in the enzymatic process. Consequently, a search was undertaken for model compounds which might more closely approximate the CO_2~enzyme-biotin complex.

It is well known that compounds with the $N\text{-}\overset{O}{\overset{\|}{C}}\text{-}O^{\ominus}$ group are very unstable and undergo decarboxylation with extreme ease, except in basic solution. However, it has long been known that certain amines absorb carbon dioxide, and in a few cases the structure of these products have been determined. For example, when ethylenediamine was allowed to react with carbon dioxide, a mixture of products was obtained whose structures were shown to be VI and VII (Katchalski et al., 1951).



VI

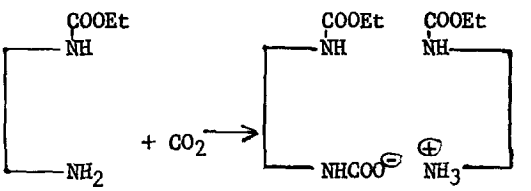


VII

Because of the structural similarity of VII to III, we became interested in this reaction and attempted to separate the products by fractional crystallization. However, the only product that we were able to identify was VI. In order to determine if the $COO^{\ominus}$ group of VI was an active group; i.e., capable of being transferred to a nucleophilic

reagent, VI was allowed to react with morpholine at room temperature. Treatment of the reaction mixture with an excess of diazomethane, followed by the usual processing gave a 46% yield of N-methoxycarbonyl-morpholine. These results establish that the carboxyl group of VI does transfer to a nucleophilic reagent under extremely mild conditions.

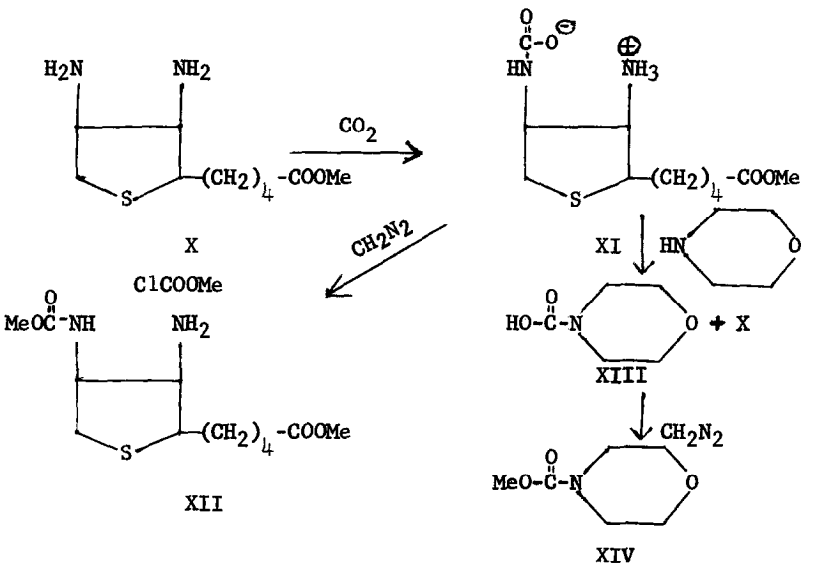
In an attempt to prepare an intramolecular salt, rather than an intermolecular salt as in VI, of ethylenediamine, we deactivated one of the nitrogens of ethylenediamine by the formation of an N-ethoxycarbonyl-ethylenediamine (VIII). When VIII was allowed to react with carbon dioxide, it also formed the intermolecular salt IX. It was also observed that when IX was exposed to the nucleophilic reagent, morpholine, the carboxyl group was transferred with the formation of N-carboxymorpholine which was isolated as its methyl ester.



VIII IX

Our data thus establish that the carboxyl group in compounds related to III will undergo transfer reactions with extreme ease, but in the cases studied thus far, the complexes were of an intermolecular nature. Our results also suggested that in order to prepare an intramolecular complex, it would be necessary to employ a 1,2-diamine in which the two amino groups were constrained to a rather restricted area in space, for example a cis-1,2-diaminocyclopentane or a cis-3,4-diaminotetrahydrothiophene. Such a stereochemical system is, in fact, present in biotin. Consequently biotin was hydrolyzed to the known diaminobiotin(Hoffmann et al., 1941). Because of solubility problems, the CO_2 complex of diaminobiotin was difficult to isolate. Therefore the methyl

ester of diaminobiotin (X) was employed; when it was exposed to carbon dioxide, a good yield of the CO_2 complex (XI) was obtained. This product was shown to be a monocarboxylated product by allowing it to react with diazomethane which caused the formation of XII in good yields. Compound XII was also prepared by allowing X to react with methyl chloroformate. The nitrogen to which the methoxycarbonyl group is attached in XII has not, as yet, been determined but has tentatively been assigned to the N_1-nitrogen on the basis of the work of Knappe et al., (1961) on a similar reaction on biotin. Further work on the placement of the methoxycarbonyl is in progress.



When the CO_2 complex (XI) was treated with morpholine, transfer of the carboxyl group of XI occurred resulting in the regeneration of the methyl ester of diaminobiotin (X) and in the formation of N-carboxy-morpholine (XIII) which was isolated as its methyl ester (XIV). Thus, a chemical model related to III has been prepared which has been shown to transfer its carboxyl group under extremely mild conditions. It appears that the CO_2 complex can be an internally stabilized salt as shown in XI and does not necessarily required stabilization from the

protein chain as indicated by Waite and Wakil (1963) in structure III. Further studies on the reactivity of XI and attempts to cause the transfer of the carboxyl group of XI to a nucleophic carbon atom are in progress.

Acknowledgment

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