D-Carbobenzoxyalanine (II) (13) was converted to the corresponding anilide (III), m.p. 162–164°, $[\alpha]_D^{30°}$ +28.4° (c 5% in HOAc), by the action of dicyclohexylcarbodiimide.

Anal.—Calcd. for $C_{17}H_{19}N_2O_3$: C, 67.96; H, 6.38; N, 9.32. Found: C, 68.19; H, 6.13; N, 9.66.

Reduction of III with lithium aluminum hydride in tetrahydrofuran afforded the hygroscopic diamine (IV), m.p. 35–36.5°, b.p. 67–71° (0.1 mm.), $[\alpha]_D^{30°}$ −29.2° (c 5% in ethanol). This compound was analyzed as the dipicrate, m.p. 143–144.5°.

Anal.—Calcd. for $C_{22}H_{22}N_8O_{14}$: C, 42.43; H, 3.56; N, 18.00. Found: C, 42.49; H, 3.60; N, 18.23.

The DL-alkylene diamine (V), m.p. 42–44°, was prepared according to the procedure of Wright, Brabander, and Hardy (11, 12). This compound was previously reported as an oil. Optical resolution of V was accomplished *via* its *d*-bitartrate salt. Five recrystallizations from ethanol afforded a bitartrate, m.p. 101–103°, of high purity. Treatment of the bitartrate with aqueous sodium hydroxide regenerated the resolved diamine (V), m.p. 59–61°, $[\alpha]_D^{27°}$ + 31.2° (c 5% in ethanol). The compound formed a monopicrate, m.p. 168–169°.

Anal.—Calcd. for $C_{23}H_{25}N_5O_7$: C, 57.11; H, 5.21; N, 14.48. Found: C, 56.84; H, 5.33; N, 14.38.

Catalytic hydrogenolysis of the resolved (+)-diamine (V) with palladium-on-carbon catalyst at 40 p.s.i. produced diamine IV, $[\alpha]_D^{27°}$ −29.5°, m.p. 34–35°. The infrared spectrum, gas chromatographic retention time, and dipicrate all were identical with those of diamine IV derived from D-alanine.

Treatment of the D-(+)-diamine (V) with propionic anhydride afforded the D-(−)-enantiomer of I, $[\alpha]_D^{27°}$ −45.7° (c 5% in ethanol), b.p. 152–157° (0.3 mm.). This compound exhibited an infrared spectrum and retention time which is identical with racemic I (11). Wright, Brabander, and Hardy (14) have independently prepared the (+)- and (−)-enantiomers of I. Our physical constants are in agreement with their data.

The analgesic potency ratio between the D- and L-forms will be the subject of a future communication.

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PHILIP S. PORTOGHESE
DENNIS L. LARSON†

Department of Pharmaceutical Chemistry
College of Pharmacy
University of Minnesota
Minneapolis 14, Minn.

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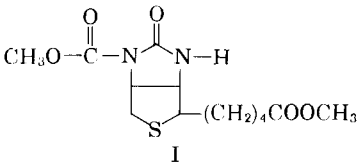
Studies on the Mechanism of Biotin Action

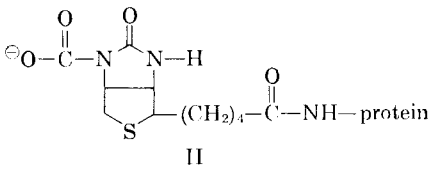
Sir:

Biotin has been implicated as an essential factor in a wide variety of carboxylation reactions in biosynthetic pathways, but the exact mechanism by which biotin functions remains unknown. For example, in the biosynthesis of purines, it has been suggested (1) that biotin is required in the carboxylation of 5-aminoimidazole ribotide to 5-aminoimidazole 4-carboxylic acid ribotide, which after several further reactions is converted into inosinic acid. Also, in the preliminary steps of fatty acid synthesis, it has been shown that a biotin-containing enzyme is involved in carboxylation of acetyl CoA to malonyl CoA (2). Studies of fatty acid synthesis in certain cell-free extracts have demonstrated that carbon dioxide is activated by a biotin-containing enzyme and that the activated $CO_2 \sim$ biotin-enzyme complex acts as the carboxylating reagent (3). It is unlikely that the carboxyl group of biotin is transferred in this carboxylation reaction since it is known that biotin is covalently bound to protein, probably through an amide linkage to the ϵ-amino group of lysine (4). We have felt that the most logical manner in which carbon dioxide could be activated by biotin is *via* one of the nitrogens of the imidazolidine ring. Recently, evidence has ac-

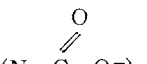
cumulated (3) concerning a biotin-containing enzyme which can utilize free D-biotin as its substrate to give an unstable product which could not be directly characterized but, after treatment with diazomethane, gave the methyl ester of N-carbomethoxybiotin (I) (5). This observation has led to the suggestion (5) that the chemical structure of the $CO_2 \sim$ biotin-enzyme complex may be represented by II.



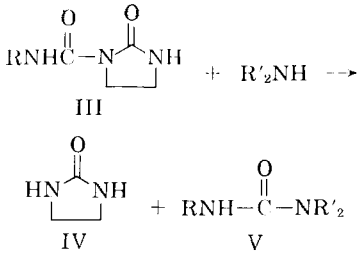
I



II

Compounds with structure  related to II are known to be very unstable and undergo decarboxylation with extreme ease. Therefore, it is probable that the actual structure of the $CO_2 \sim$ biotin-enzyme complex is not one in which the carboxylate anion is present as such but rather one in which the carboxylate anion of biotin has been partially stabilized by reaction with the associated protein. Thus, we visualize these carboxylation reactions as involving the following steps: (*a*) formation of a $CO_2 \sim$ biotin-enzyme complex, (*b*) temporary stabilization of the complex by reaction of the carboxylate anion with a functional group of the associated protein, such as an amino or hydroxyl group, and (*c*) transfer of the carboxyl group to an attacking nucleophilic agent, which would produce the corresponding carboxylic acid and regenerate the biotin-containing enzyme.

In order to gain more information about the mechanism of this reaction, we have undertaken a study of the transfer of a carbonyl group to an attacking nucleophilic reagent using model compounds related to the proposed $CO_2 \sim$ biotin-enzyme complex; *i.e.*, we have studied step (*c*) of the biochemical carboxylation reaction. Because of the instability of compounds with the general structure II, it was necessary to use model compounds which would not undergo spontaneous decarboxylation. The simplest model systems for such a study are the N-substituted derivatives of 2-imidazolidone (III).



III IV V

The model compounds which we chose for our initial study were those in which *R* in III was *p*-methoxyphenyl, phenyl, or *p*-nitrophenyl, and the nucleophilic reagents were morpholine or piperidine.

In a typical experiment, 2-imidazolidone was allowed to react with *p*-methoxyphenyl isocyanate to give a good yield of N-*p*-methoxyphenylcarbamyl-2-imidazolidone (m.p. 175–176°).

Anal.—Calcd. for $C_{11}H_{13}N_3O_3$: C, 56.11; H, 5.52; N, 17.86. Found: C, 56.46; H, 5.28; N, 17.86.

When N-*p* methoxyphenylcarbamyl-2-imidazolidone was allowed to react with morpholine at reflux temperature, the model compound was cleaved to give 2-imidazolidone (IV) and the corresponding urea (V) (m.p. 124–125° in agreement with the literature value (6)).

These reactions represent examples of the transfer of a carbonyl group from model compounds, which are related to the proposed $CO_2 \sim$ biotin-enzyme complex, to a nucleophilic reagent. Although the rate of these reactions is relatively low, experiments with other model compounds and other nucleophilic reagents are in progress in which we hope to observe more facile transfer of the carbonyl group. Nevertheless, the transfer of carbonyl groups in model compounds offers support to the proposed mechanism of action of biotin involving the complex represented by II.

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HOWARD J. SCHAEFFER
PARMATMA S. BHARGAVA

Department of Medicinal Chemistry
School of Pharmacy
The University of Buffalo
Buffalo, New York

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