that it was dependent on the conformation of the asymmetric center. The concepts derived from this work have been applied, with success, to the correlation of configuration.[4] These studies involved addition reactions to a carbonyl function which was located adjacent to or near an asymmetric center. Asymmetric syntheses by the addition to an olefinic moiety in both catalytic[5] and non-catalytic[6] reactions also have been observed.

We wish to report that the addition of diphenyldiazomethane to (−)-menthyl acrylate (I, R = H) and (−)-menthyl methacrylate (I, R = CH_3) results in partial asymmetric synthesis.

When 1.48 g. (0.0076 mole) of diphenyldiazomethane was added slowly to 1.61 g. (0.0076 mole) of (−)-menthyl acrylate[7] an exothermic reaction accompanied by immediate decolorization and evolution of nitrogen occurred. In order to avoid any possibility of the resolution of the diastereoisomers that are formed, isolation of products at this point was avoided. The reaction mixture was saponified by refluxing with a solution of 0.85 g. of potassium hydroxide in 60 ml. of ethylene glycol for 52 hours to assure completeness.[8] The reaction mixture was diluted with water and extracted 4–5 times with ether. The residue from the ether extract showed less than 0.1% of carbonyl absorption at 1720 cm.$^{-1}$. The aqueous fraction was acidified with hydrochloric acid and yielded 1.08 g. (60%) of 2,2-diphenylcyclopropanecarboxylic acid (II, R = H), m.p. 163–164.5°, whose infrared spectrum was identical in all respects with an authentic sample[9] and which had a rotation of $[\alpha]^{24}D$ −4.7° ($CHCl_3$). This corresponds to 2.2% of asymmetric synthesis.

The above procedure was repeated using (−)-menthyl methacrylate[7,10,11] (I, R = CH_3) which after complete saponification yielded 1-methyl-2,2-diphenylcyclopropanecarboxylic acid (II, R = CH_3) in 74% yield, m.p. 176–180.5°, whose infrared spectrum was identical with that of an authentic sample.[12] The rotation of $[\alpha]^{24}D$ +3.7° ($CHCl_3$) corresponds to 10% asymmetric synthesis.

It has been demonstrated that the addition of diazoalkanes to olefins proceeds in a stereospecific manner.[13] The resulting Δ′-pyrazolines in turn decompose stereospecifically.[13] The asymmetric synthesis can be visualized as proceeding by an attack of the diazoalkane predominantly in the direction indicated by the arrow in Fig. 1 to yield

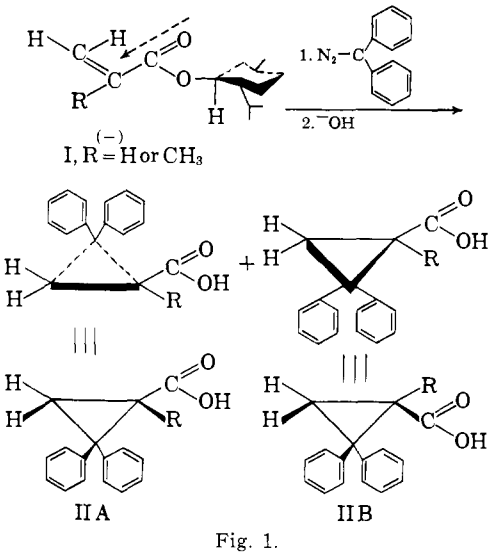
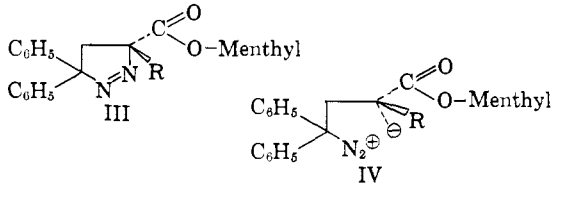


Fig. 1.

III in excess, which decomposes to yield a predominance of IIA. An alternative is the formation of a zwitterion intermediate IV which can collapse directly to product rather than proceed through III. That this reaction proceeds via a diphenyl-



methylene (carbene) addition[14] has been ruled out provisionally in this case[15] on the observation that the copper-catalyzed addition of diphenyldiazomethane to (−)-menthyl acrylate yielded totally inactive product.

On the basis of Prelog's[4] correlation we have assigned the D-configurations to (−)-IIA (R = H) and to (+)-IIA (R = CH_3). The detailed mechanism of the addition of various diazoalkanes to olefins and its application to the establishment of absolute configuration of other cyclopropanecarboxylic acids is currently under investigation.

(5) M. G. Vavon and B. Jakubowicz, *Bull. Soc. Chim.*, **53**, 1111 (1933); D. Lipkin and T. D. Stewart, THIS JOURNAL, **61**, 3295 (1939); C. L. Arcus and D. G. Smyth, *J. Chem. Soc.*, 35 (1955).

(6) M. P. Balfe, J. Kenyon and D. Y. Waddan, *ibid.*, 1367 (1954).

(7) C. S. Marvel and R. L. Frank, THIS JOURNAL, **64**, 1675 (1942).

(8) The use of alcoholic potassium hydroxide or shorter reaction time resulted in incomplete saponification as shown by the appearance of a carbonyl band at 1720 cm. $^{-1}$ in the infrared spectrum of the neutral fraction from the saponification. Incomplete saponification would be equivalent to partial resolution.[3]

(9) H. M. Walborsky and F. M. Hornyak, *ibid.*, **77**, 6026 (1955).

(10) C. E. Rehberg, M. B. Dixon and C. H. Fisher, *ibid.*, **67**, 210 (1945).

(11) We wish to thank Mr. A. Young for the preparation of this ester.

(12) H. M. Walborsky and F. J. Impastato, *Chem. and Ind.*, 1690 (1958).

(13) K. V. Auwers, *et al.*, *Ber.*, **66**, 1198 (1933); *Ann.*, **470**, 284 (1924); **496**, 252 (1932); J. van Alphen, *Rec. Trav. Chim.*, **62**, 210 (1943).

(14) P. S. Skell and A. Y. Garner, THIS JOURNAL, **78**, 5430 (1956); W. von E. Doering and P. M. LaFlamme, *ibid.*, **78**, 5447 (1956).

(15) Whether other methylenes will yield asymmetric synthesis is under investigation.

(16) To whom inquiries regarding this work should be sent.

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RECEIVED FEBRUARY 2, 1959

ENZYMATIC SYNTHESIS OF CARNOSINE FROM β-ALANYL ADENYLATE AND HISTIDINE

Sir:

Previous studies have provided evidence for enzymatic formation of amino acyl adenylates[1,2]; however, proof that such anhydrides are inter-

(1) M. Karasek, P. Castelfranco, P. R. Krishnaswamy and A. Meister, THIS JOURNAL, **80**, 2335 (1958).

(2) H. S. Kingdon, L. T. Webster and E. W. Davie, *Proc. Nat. Acad. Sci. (U.S.)*, **44**, 757 (1958).

mediates in biological peptide bond synthesis is lacking. We report here enzyme-catalyzed synthesis of carnosine (β-alanyl-L-histidine) from β-alanyl adenylate and histidine. Carnosine synthesis from β-alanine and L-histidine was observed in the presence of ATP, Mg^{++} and soluble enzyme preparations obtained from chicken and rabbit skeletal muscle,[3] by extraction of the muscle with

TABLE I

Exp.	Reaction mixtures[a]	C^{14}-Carnosine (c.p.m.)[e]
1	C^{14}-β-Alanine + L-histidine + ATP[b]	13,100
2	β-Alanine + ATP + C^{14}-DL-histidine[b]	12,900
3	β-Alanyl-adenylate[c] + C^{14}-DL-histidine	15,800
4	β-Alanyl-adenylate + C^{14}-DL-histidine[d]	2,750

[a] Composition of reaction mixtures; Exp. 1. β-alanine-1-C^{14} (250,000 c.p.m., 0.08 micromole), L-histidine (3 micromoles), ATP (2.5 micromoles), $MgCl_2$ (3 micromoles), KCl (100 micromoles), sodium phosphate buffer (67 micromoles) and enzyme (10 mg.); *p*H 7.4 final volume, 1 ml.; incubated for 20 min. at 37°. Exp. 2–4. β-alanine (3 micromoles), ATP (2.5 micromoles), β-alanyl adenylate (3.8 micromoles), DL-histidine-2-C^{14} (0.8 micromole), 416,000 c.p.m.); no $MgCl_2$ or KCl was added in exp. 3 and 4; other conditions were as in exp. 1. [b] No carnosine was formed when any one of these components, $MgCl_2$, or enzyme was omitted, or when guanosine triphosphate, cytidine triphosphate, uridine triphosphate, or inosine triphosphate was used at equivalent concentrations in place of ATP. Substitution of equimolar concentrations of $CoCl_2$ or $MnCl_2$ for $MgCl_2$ gave less than 25% of the reported activity. [c] No carnosine was formed when β-alanyl-adenylate was hydrolyzed prior to use. [d] Enzyme was omitted or heated at 100° for 10 min. prior to use. [e] Separated by ascending paper chromatography on Whatman No. 3 paper and a solvent consisting of ethanol, acetic acid, H_2O (75:5:20); other solvents, *e.g.*, butanol, formic acid, H_2O (75:15:10), phenol, H_2O (80:20; saturated with HCl), gave similar results.

a solution containing 0.65 *M* NaCl and 0.04 *M* $KHCO_3$, and then fractionation with ethanol at −5 to −10°. Formation of C^{14}-carnosine from C^{14}-β-alanine or C^{14}-histidine was demonstrated by paper chromatography in several solvents; the C^{14}-carnosine was eluted and hydrolyzed with hydrochloric acid or carnosinase[4] to yield the C^{14}-precursors. No carnosine was formed when histidine, β-alanine, Mg^{++}, ATP, or enzyme were separately omitted. Substitution of equivalent quantities of D-histidine[5] for L-histidine gave less than 5% of the radioactivity in the carnosine area, while a compound with the properties of anserine (β-alanyl-1-methyl-L-histidine) was formed when histidine was replaced by 1-methylhistidine. Addition of KCl was required for optimum activity with certain enzyme fractions; added coenzyme A (0.002 *M*) did not affect the rate of synthesis. Addition of pyrophosphatase (50 micrograms per ml.)[6] increased synthesis by about 25%.

When β-alanyl adenylate, synthesized as previously described,[7] was added to the reaction mixture in place of ATP, Mg^{++}, KCl, and β-alanine, considerable synthesis of carnosine was observed (Table I). Under these conditions some radioactivity appeared in the carnosine area in the absence of enzyme. Enzymatic formation of carnosine was not observed in experiments with β-alanyl phosphate. The results suggest that carnosine synthesis involves activation of β-alanine to yield β-alanyl adenylate, and reaction of this anhydride with histidine to form carnosine. Mg^{++} ions appear to be required only for activation. In analogy with tryptophanyl adenylate,[1] β-alanyl adenylate may be tightly bound to enzyme, and therefore studies with relatively large amounts of purified enzyme may be required for isolation of enzymatically-formed β-alanyl adenylate.[8]

(3) While this work was in progress, T. Winnick and R. E. Winnick (Abstracts, 4th International Congress of Biochemistry, Vienna, 1958) reported synthesis of carnosine from β-alanine and histidine by chick muscle preparations.

(4) H. T. Hanson and E. L. Smith, *J. Biol. Chem.*, **179**, 789 (1949).

(5) Generously donated by Dr. Jesse P. Greenstein.

(6) L. A. Heppel, in "Methods in Enzymology" (Colowick and Kaplan, eds.) **2**, 570 (1955).

(7) P. Castelfranco, K. Moldave and A. Meister, THIS JOURNAL, **80**, 2335 (1958); "Microsomal Particles and Protein Synthesis" (R. B. Roberts, ed.), Wash. Acad. Sci., 115 (1958).

(8) We wish to thank the National Heart Institute (National Institutes of Health) of the Public Health Service and the National Science Foundation for generous support of this research; abbreviation ATP, adenosine triphosphate.

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RECEIVED JANUARY 22, 1959

AMPEROMETRIC DETERMINATION OF FLUORIDE AT A ROTATED ALUMINUM ELECTRODE

Sir:

From 99.994% pure aluminum wire (1/16 inch diameter) a rotated electrode (600 r.p.m.) has been constructed of about 0.15 $cm.^2$ surface area. After each set of measurements it was "cleaned" in a 0.01 *M* ethylenediaminetetraacetate buffer of *p*H 3. All experiments were carried out at 25°. In nitric acid solutions of *p*H 2 or less and acetate buffers of *p*H 3 to 5.5 the electrode is highly polarized. Anodic depolarization occurs at about +0.6 volt and cathodic depolarization at −1.4 volt (*vs.* S.C.E.). Perchlorates and halides exert an anodically depolarizing effect at about −0.6 volt, but they do not depolarize cathodically.

In acid medium the zero current potential is ill-defined and extremely poorly reproducible. Fluoride in acid medium depolarizes the electrode anodically, it causes the potential to become extremely negative. Current–potential curves in the presence of fluoride are of a composite nature. In the absence of oxygen anodic limiting currents are found at −0.75 volt which are proportional to the fluoride concentration in a range between 10^{-5} and 3×10^{-4} *M*. In the amperometric determination of fluoride in an oxygen-free acetate buffer of *p*H 3.5 use is made of the "standard addition" method which makes the accuracy independent of the reproducibility of the surface conditions of the electrode. Alkali perchlorates, nitrates, chlorides, sulfates and 0.002 *M* calcium do not interfere. Phosphate decreases the proportionality factor between limiting current and concentration but *does not interfere*. The results of the amperometric method have been tested in Minneapolis city water and eight natural waters and good agreement with results obtained by classical methods has been obtained. Baker and Morrison[1] determined microgram quantities of fluoride by spontaneous elec-

(1) B. B. Baker and J. D. Morrison, *Anal. Chem.*, **27**, 1306 (1955).