removed in the course of the reaction) but also a potential method for relating the absolute configurations of allenes to the configurations of their cyclopropane progenitors.[3]

The optically active nitrosourea[4] (I) was synthesized from optically active *trans*-2,3-diphenylcyclopropane carboxylic acid[5] by the same reaction scheme used for the synthesis of the 2,2-diphenyl analog.[1]

Treatment of the nitrosourea with lithium ethoxide (alcoholate) in heptane at 0° gave a hydrocarbon-soluble material that showed an ultraviolet spectrum that was identical with that of pure, racemic 1,3-diphenylallene prepared by the method of Jacobs and Danker.[6] From the ultraviolet spectrum, the yield of allene was calculated to be 79.2%. Evaporation of the hydrocarbon-soluble fraction gave a pale yellow granular solid, m.p. 35–42°. The infrared spectrum (KBr) of this material was identical with that of pure racemic material (KBr) except for the presence of a low intensity broad absorption centering at about 5.9 μ and the absence of a distinct peak at 11.3 μ (overlapping with a peak at 11.4 μ). Assuming the crude pale yellow solid is pure allene, the material showed $[\alpha]^{25}D$ +419° (ethanol). Recrystallization of this material from pentane gave white needles, m.p. 52–56°; $[\alpha]^{24}D$ +797° (ethanol).[7] The infrared (KBr) and ultraviolet spectra of this material were identical with the corresponding spectra of pure racemic 1,3-diphenylallene except between 11.0 and 11.5 μ.

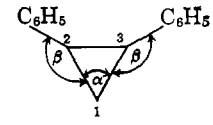
The precursor or precursors to the allene have not yet been conclusively determined. However, by analogy to the base-induced decomposition of N-nitroso-N-(2,2-diphenylcyclopropyl)urea,[1] the reaction scheme most likely involves initial formation of the diazocyclopropane that can either decompose in a concerted fashion to the allene or proceed first to the cyclopropylidene that can then collapse to give the allene.

It is naturally of interest to speculate as to the origin of the optical activity in the allene as well as to attempt to examine what the retention of asymmetry means with regard to the more intricate details of the mechanism of formation of the allene from its precursors. Some insight into both of these problems can be gained by considering individually the various structural and geometrical changes that must occur during the conversion of either the diazocyclopropane or the cyclopropylidene to the allene. Four changes of real significance must take place. The bond between C_2 and C_3 (Scheme I) must break, the β-angle must increase from about 150 to 180°, the α-angle must increase from 60 to 180°, and, finally, planes A and B (Scheme II) must rotate 90° with respect to each other.

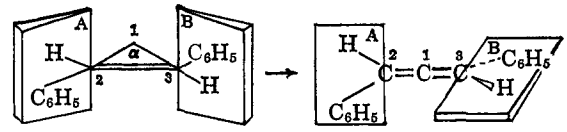
Now, if the α- and β-angles increase to 180° before planes A and B rotate, the allene must be inactive, since this sequence entails a symmetrical intermediate. Thus, the mere fact that the allene is optically active demonstrates unequivocally that rotation of planes A and B must at least begin before α and β increase to 180°.

Furthermore, the fact that rotation of the two planes precedes collinearity of carbons 1, 2, and 3 immediately suggests that the source of the optical activity of the allene resides in steric effects that affect the direction of rotation of the planes A and B. Thus,

SCHEME I



SCHEME II



rotation of plane A or plane B in a counterclockwise direction leads to one enantiometric allene. However, either of these changes brings a phenyl group into opposition with the phenyl and hydrogen described by the other plane. On the other hand, rotation of plane A or plane B in a clockwise direction leads to the other enantiomer and brings a hydrogen in one plane into opposition with the phenyl and hydrogen in the other plane. This analysis suggests that this system might well be useful for relating the absolute configurations of optically active allenes with their cyclopropane precursors. However, the situation is not quite as straightforward as it appears at first glance. Thus, the major portion of the optically active allene might well result from the concerted collapse of a diazocyclopropane in which a nitrogen molecule partially bonded to C-1 could conceivably present a steric effect that is in opposition to the effect described before. We are presently engaged in experiments that we hope will clarify this point and will demonstrate whether or not this is truly a potentially general method to relate the absolute configurations of allenes to optically active cyclopropane acids.

Acknowledgment.—The authors are indebted to the National Science Foundation and to the U. S. Army Research Office (Durham) for their generous support of this work.

(3) For references to configurational correlations between optically active allenes and their nonallenic precursors, see ref. 2, pp. 315–316.

(4) Correct analyses were obtained for all new optically active compounds except for the nitrosourea which was unstable enough at room temperature to preclude sending away for analysis.

(5) L. A. D'yakonov, M. I. Komendantov, Fu Gui-siya, and G. L. Korichev, *J. Gen. Chem. USSR*, **32**, 917 (1962).

(6) T. L. Jacobs and D. Danker, *J. Org. Chem.*, **22**, 1424 (1957).

(7) 1,3-Diphenylallene of low optical purity has been previously prepared by Jacobs and Danker[6] ($[\alpha]^{28}D$ +2.48; −1.24° (CCl_4)).

(8) Alfred P. Sloan Fellow.

(9) N.S.F. summer research participant, 1962.

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Enzymatic Stereospecifity in the Dehydrogenation of Stearic Acid to Oleic Acid[1]

Sir:

The enzyme-catalyzed conversion of stearic acid to oleic acid (*cis*-Δ^9-octadecenoic acid) shows positional and geometrical specificity. We now have found that in *Corynebacterium diphtheriae* this enzyme system has the further property of selectively removing one particular hydrogen from each pair of hydrogens at carbon atoms 9 and 10 of the polymethylene chain.

This investigation has been made possible by the finding that methyl 9-hydroxyoctadecanoate,[2] prepared by catalytic hydrogenation of the naturally occurring Δ^{12}-9-hydroxyoctadecenoic acid,[3] is optically active and has the same sign of rotation as synthetic methyl 9D-hydroxyoctadecanoate.[4] We have also

(1) Supported by grants-in-aid from the National Science Foundation, the United States Public Health Service, the Life Insurance Medical Research Fund, and the Eugene Higgins Trust Fund of Harvard University.

(2) A generous gift from Dr. A. J. Fulco.

(3) F. D. Gunstone, *J. Chem. Soc.*, 1274 (1952).

(4) We are indebted to Professor Gunstone for a gift of synthetic 9D-hydroxyoctadecanoic acid. Our finding that both the "natural" and the synthetic samples are levorotatory confirms the assignment of the D-configuration to the "natural" acid made by Baker and Gunstone on the basis of mixture melting point data: C. D. Baker and F. D. Gunstone, *J. Chem. Soc.*, 759 (1963).

shown that the methyl ester of a 10-hydroxyoctadecanoic acid, obtained by the action of a *pseudomonas* species on oleic acid,[5] is optically active.

The methyl esters of the two optically active hydroxy fatty acids were converted to their enantiomorphs by treatment of their tosylates with base.[6] Hydrogenolysis of the tosylates of each of the four optically active hydroxy fatty acid methyl esters (9L, 9D, and 10L, 10D) with tritium-labeled lithium aluminum hydride yielded, after chromic acid oxidation of the resulting octadecanols, the four desired tritium-labeled stearic acids.[7] The chemical purity of the fatty acid samples as determined by v.p.c. was greater than 99%. The radiopurity, as judged by vapor phase chromatographic and isotope dilution techniques, was greater than 90% in all cases. Each of the four labeled stearic acids was mixed with a small amount of stearic acid 1-C^{14} and incubated with growing cultures of a nontoxin-producing strain of *Corynebacterium diphtheriae*.[8] Under the conditions used, up to $^2/_3$ of the stearate was converted to oleate and no significant degradation of fatty acid occurred.[9] The total cellular fatty acids were then isolated as the methyl esters and separated into saturated and unsaturated esters by treatment with mercuric acetate followed by chromatography on silicic acid columns.[10] Methyl oleate[11] and methyl stearate were isolated by v.p.c. Simultaneous assay of tritium and C^{14} was carried out in a liquid scintillation spectrometer. The results of one representative experiment are shown in Table I. The data obtained with the two 9-tritio acids indicate that the hydrogen in the D-position of stearate was removed during the formation of oleate while that in the L-position was not. The recovered methyl stearate remaining after the incubation of the 9D-isomer was considerably enriched with respect to tritium; $H^3:C^{14} = 1.6$ as compared to 1.0 in the original substrate. This finding indicates a primary kinetic isotope effect and suggests that the removal of the 9D-hydrogen is a rate-limiting step in the over-all reaction. Abstraction of hydrogen from C-10 on conversion to oleate was also stereospecific, since it occurred only with one of the two isomers.[12] In this case the methyl stearate recovered after incubation was not enriched with respect to tritium, and we therefore infer from the absence of a significant isotope effect that the removal of hydrogen at C-10 is not a rate-limiting step in the over-all desaturation reaction.

TABLE I

ENZYMATIC DESATURATION OF H^3, C^{14}-STEARIC ACIDS LABELED STEREOSPECIFICALLY WITH TRITIUM

	H^3C^{14} Ratios in Stearate and Oleate Recovered after Incubation[a]	
Substrate	Methyl stearate	Methyl oleate
9D-H^3-stearic acid-1-C^{14}	1.58	0.35
9L-H^3-stearic acid-1-C^{14}	1.02	.89
10-H^3-stearic acid-1-C^{14} (prepared from (+) 10-hydroxyoctadecanoate)	0.99	.10
10-H^3-stearic acid-1-C^{14} (prepared from (−)-10-hydroxyoctadecanoate)	.85	.87

[a] For ease of comparison, the ratios presented in this table have been calculated in reference to an assigned value of unity in the substrate, *i.e.*, measured $H^3:C^{14}$ ratio in compounds after incubation divided by the measured $H^3:C^{14}$ ratio of the substrate.

Thus, the enzymic desaturation at the 9,10 position of stearic acid is characterized by a rate-limiting, stereospecific removal of the 9D-hydrogen followed by a nonrate-limiting, stereospecific loss of one of the hydrogens at C-10. This is the first report on the stereochemistry of hydrogen elimination on introduction of an isolated double bond into an acyclic compound. It represents a notable example of the stereospecificity of enzyme-catalyzed reactions at *meso* carbon atoms.[13,14]

(5) L. L. Wallen, R. G. Benedict, and R. W. Jackson, *Arch. Biochem. Biophys.*, **99**, 249 (1962). We are indebted to Dr. Wallen for a gift of a culture of this organism.

(6) Optical activity of the methyl hydroxystearates was measured with a Rudolf spectropolarimeter at 23 ± 1° in methanol solution. At 546 mμ the compounds had the following specific rotations (± stand. dev.). I, methyl 9-hydroxyoctadecanoate from Δ^{12}-9-hydroxydecanoic acid, −0.18 ± 0.04°. II, methyl 9D-hydroxyoctadecanoate (synthetic), −0.17 ± 0.5°. III, methyl 9-hydroxyoctadecanoate from the tosylate of I, +0.17 ± 0.04°. IV, methyl 10-hydroxyoctadecanoate, produced by *pseudomonad* culture from oleic acid, −0.16 ± 0.07°. V, methyl 10-hydroxyoctadecanoate from the tosylate of IV, +0.15 ± 0.05°. The magnitude of the rotation of each sample increased with decreasing wave length.

(7) From the results of others (E. R. Alexander, *J. Am. Chem. Soc.*, **72**, 3796 (1950), and G. K. Helmkamp and B. F. Rickborn, *J. Org. Chem.*, **22**, 479 (1957)), we infer that direct replacement of the tosyl function by tritium occurred with inversion of configuration. Thus, the tosylate of methyl 9D-hydroxyoctadecanoate is assumed to yield 9L-H^3-octadecanoic acid. Both in the inversion of the hydroxyl functions and in the hydrogenolysis some racemization is to be expected. The resultant stearic acids will therefore be partially racemized with respect to tritium and the enzymatic conversion to oleic acid (Table I), even if stereospecific, will not proceed with complete removal or retention of labeled hydrogen.

(8) Kindly provided to us by Professor A. M. Pappenheimer.

(9) A. J. Fulco, R. Levy, and K. Bloch, unpublished.

(10) H. Goldfine and K. Bloch, *J. Biol. Chem.*, **236**, 2596 (1961).

(11) The identity of the methyl oleate is based on v.p.c. data, catalytic reduction to methyl stearate, degradation by permanganate–periodate,[9] and demonstration of *cis*-configuration by thin layer chromatography (L. Morris, *Chem. Ind.* (London), 1238 (1962)).

(12) We are not able to specify the absolute configuration of the hydrogen which is removed from carbon atom 10 since the necessary information for assigning configurations to the 10-hydroxyoctadecanoic acids used as starting materials is unavailable.

(13) F. H. Westheimer, H. F. Fisher, E. E. Conn, and B. Vennesland, *J. Am. Chem. Soc.*, **73**, 2402 (1951).

(14) R. H. Levy, P. Talalay, and B. Vennesland in "Progress in Stereochemistry," Vol. 3, P. B. D. De La Mare and W. Klyne, Ed., 1962, p. 299.

(15) Recipient of Research Career Development Award from the National Institutes of Health. On leave of absence from the Department of Biochemistry, University of Minnesota.

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Isolation and Identification from Common Vetch of γ-L-Glutamyl-β-cyano-L-alanine, a Bound Form of the Neurotoxin β-Cyano-L-alanine[1-3]

Sir:

Vicia sativa (common vetch), like a number of other cultivated vetches, is extensively grown and used as a forage plant and soil-improving crop.[4] Incidents of poisoning by common vetch have been noted and reviewed.[5] The recent isolation of the neurotoxic amino acid β-cyano-L-alanine from the seed of common vetch has been of added interest in connection with the implication of this seed as a contaminant in foods which have been associated with causing lathyrism in man.[6,7] However, the concentration of β-cyanoalanine (0.15%) in a contaminant

(1) Aided by U. S. Public Health Service Grant NB 04316-01 and by Muscular Dystrophy Associations of America.

(2) Presented at the 145th National Meeting of the American Chemical Society, New York, N. Y., Sept., 1963, at the Symposium on Deleterious Compounds of Natural Origin in Foods and Feeds.

(3) We thank Miss Hilda Malodeczky, Mrs. Harriet R. Levie, and Mr. Adam Zsolnay for capable technical assistance.

(4) P. R. Henson and H. A. Schoth in "Vetch Culture and Uses," Farmers' Bulletin No. 1740, U. S. Department of Agriculture, Government Printing Office, Washington, D. C., 1961; L. H. Bailey, Ed., "The Standard Cyclopedia of Horticulture," Vol. III, the Macmillan Company, New York, N. Y., 1929–1930, p. 3465.

(5) D. G. Steyn, *Onderstepoort J. Vet. Sci. Animal Ind.*, **1**, 219 (1933).

(6) C. Ressler, *J. Biol. Chem.*, **237**, 733 (1962), and references cited therein.

(7) *Nutr. Rev.*, **21**, 28 (1963).