perature and a detector temperature of 125 °C; the retention times of compounds **11**, **2**, and **9** were 2.6, 10.5, and 11.5 min, respectively. Compounds **2** and **9** showed broad, overlapping peaks, and only 90% pure **9** was isolated. Similar thermal behavior was observed for the 7-deuteriated analogues.

5-(2-Methylethyl)cyclohexa-1,3-diene (11): ^{1}H NMR δ 0.89 (d, 3 H, J = 3.5 Hz, CH_3), 0.91 (d, 3 H, J = 3.5 Hz, CH_3), 1.59-1.78 (m, 1 H, $CHMe_2$), 2.04-2.21 (m, 3 H, C5-H, C6-H), 5.67-5.92 (m, 4 H, olefinic H); MS, m/z (relative intensity) 122 (15, M^+), 79 (100), 78 (39), 77 (34).

5-(2-Methylethyl-*2-d*)cyclohexa-1,3-diene: ^{1}H NMR δ 0.89 (s, 3 H, CH_3), 0.90 (s, 3 H, CH_3), 2.04-2.20 (m, 3 H, C5-H, C6-H), 5.63-5.92 (m, 4 H, olefinic H); MS, m/z (relative intensity) 123 (18, M^+), 79 (100), 78 (42), 77 (33).

2-Methylocta-2,4(*Z*),6(*Z*)-triene-*8-d* (2-*d*): ^{1}H NMR δ 1.77 (d, 2 H, J = 5.8 Hz, CH_2D), 1.78 (s, 3 H, cis-CH_3), 1.83 (s, 3 H, *trans*-CH_3), 5.55 (apparent quartet, 1 H, C7-H), 6.12-6.33 (m, 3 H, C3,C4,C5-H), 6.48 (apparent t, 1 H, C6-H). MS, m/z (relative intensity) 123 (52, M^+), 108 (100), 92 (43), 91 (59), 79 (37).

2-Methyl-2,4(*Z*),6(*E*)-octatriene-*8-d*: ^{1}H NMR δ 1.76 (s, 3 H, CH_3), 1.79 (d, 3 H, J = 7.1 Hz, C8-H), 1.83 (s, 3 H, CH_3), 5.70 (dt, 1 H, J = 6.6 Hz, 14.3 Hz, C7-H), 5.86 (t, 1 H, J = 11 Hz, C5-H), 6.04 (t, 1 H, J = 11 Hz, C4-H), 6.27 (d, 1 H, J = 11.2 Hz, C3-H), 6.52 (t, 1 H, J = 13 Hz, C6-H); MS, m/z (relative intensity) 123 (54, M^+), 108 (100), 92 (42), 91 (58), 79 (37).

Kinetic Measurements. Kinetics of the thermal isomerizations were measured with ca. 1% solutions of trienes in 2-methylpentane. Typically 16 μL of the triene solution was placed in each of a series of 0.5-mm capillary tubes, cooled to -78 °C, and sealed either directly or after establishing an argon atmosphere. These ampules were heated in an oil bath fitted with a mechanical stirrer, heating elements controlled by a Model 253 Bayley precision temperature controller, a metal wire basket for holding the tubes, and a Hewlett-Packard 2802A digital thermometer. The temperature of the bath was maintained to ±0.02 °C. Samples were withdrawn at appropriate time intervals, cooled in liquid nitrogen or dry ice-acetone, and analyzed by integrating peaks at 4.17 min (**1** or **1-*d***) and at 7.73 min (**2** or **2-*d***) on the phenyl methyl silicone capillary GC column. Each thermolysis reaction mixture was analyzed at least three times. The averaged values are reported in Table I.

Acknowledgment. We are indebted to the National Science Foundation for support of our work on hydrocarbon rearrangements, and to Professors B. A. Hess, Jr., M. M. Kreevoy, Y. Mazur, W. H. Saunders, Jr., and L. J. Schaad for helpful discussions and correspondence.

Communications to the Editor

Biosynthesis of the Unusual Amino Acid 5-Hydroxy-4-oxonorvaline

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Received July 5, 1988

Non-protein amino acids, as a group of natural products, possess a wide array of chemical structures and biological activities, but extensive biosynthetic investigations are limited to a few members of this group of unusual amino acids.[1] The hydroxyketone-containing amino acid, 5-hydroxy-4-oxonorvaline (HON, **3**),[2] possesses antitubercular[3] and antifungal[4] properties, and we now report results which demonstrate that the initial step in the biosynthetic formation of this unusual amino acid is analogous to the proposed initial step in the biosynthesis of carbapenem antibiotics.[5]

In a typical experiment (Figure 1), an aqueous solution of ^{13}C-labeled substrate (8 mmol) was administered in two equal portions (one at the onset of HON production[6] and the second 24 h later) to *Streptomyces akiyoshiensis* (ATCC 13480) in 500 mL of medium[8] containing starch and Pharmamedia. After an additional 24 h of incubation, the cells were removed by centrifugation, and HON (ca. 2 mmol) was obtained from the resulting culture broth in one of two ways.[2] In experiments 1, 2, and 4, HON was isolated directly from the charcoal-treated culture broth by cation exchange chromatography (Amberlite IR-120) and separated from acidic amino acids by anion exchange chromatography (Dowex 1-X8). For further purification and subsequent ^{13}C NMR analysis, HON was converted by $NaIO_4$ cleavage to aspartate and formaldehyde (isolated as its dimethone derivative).[9] For experiments 3 and 5, in which doubly labeled precursors were used, $NaBH_4$ was added to the culture broth to reduce HON to a mixture of two diastereomers of 4,5-dihydroxynorvaline which were isolated by ion-exchange chromatography and converted in concentrated HCl to a corresponding mixture of diastereomeric γ-lactone hydrochloride salts[2] for purification by recrystallization and ^{13}C NMR analysis.

The results of five separate feeding experiments with sodium [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]acetates[10] and DL-[4-^{13}C]-[10] and DL-[2-^{13},^{15}N]aspartates are presented in Figure 1. The pattern of ^{13}C enrichment and ^{13}C-^{13}C coupling observed in HON, obtained from the three experiments which used labeled acetates as substrates, showed that C-1 to C-4 of HON are derived from a 4-carbon intermediate of the citric acid cycle and that C-5 is derived directly from the methyl carbon of acetate. The nature of the 4-carbon precursor was probed by feeding DL-[4-^{13}C]aspartate. The principal incorporation of ^{13}C label (3.4 times natural abundance) into C-4 of HON demonstrated that oxaloacetate, malate, or aspartate, and not a symmetrical 4-carbon intermediate of the citric acid cycle, serves as the 4-carbon precursor to HON. The smaller ^{13}C enrichment (1.5 times natural abundance) observed at C-1 of HON would be expected if a portion of the administered DL-[4-^{13}C]aspartate had been converted to a symmetrical citric acid cycle intermediate (e.g., fumarate) either via oxaloacetate and malate or directly by the action of aspartate ammonia lyase.[11] Aspartate, synthesized from fumarate formed

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(9) The four carbons of aspartate correspond to C-1 to C-4 of HON, respectively, and the carbon in formaldehyde corresponds to C-5 of HON. ^{13}C Enrichments were calculated for the carbon derived from HON in formaldehyde dimethone, relative to the natural abundance ^{13}C signals provided by the dimedone reagent, and for the carbons in aspartate, relative to the carboxyl signal of disodium malonate which was added as in internal standard.
(10) Obtained from MSD Isotopes, Montreal, Canada.

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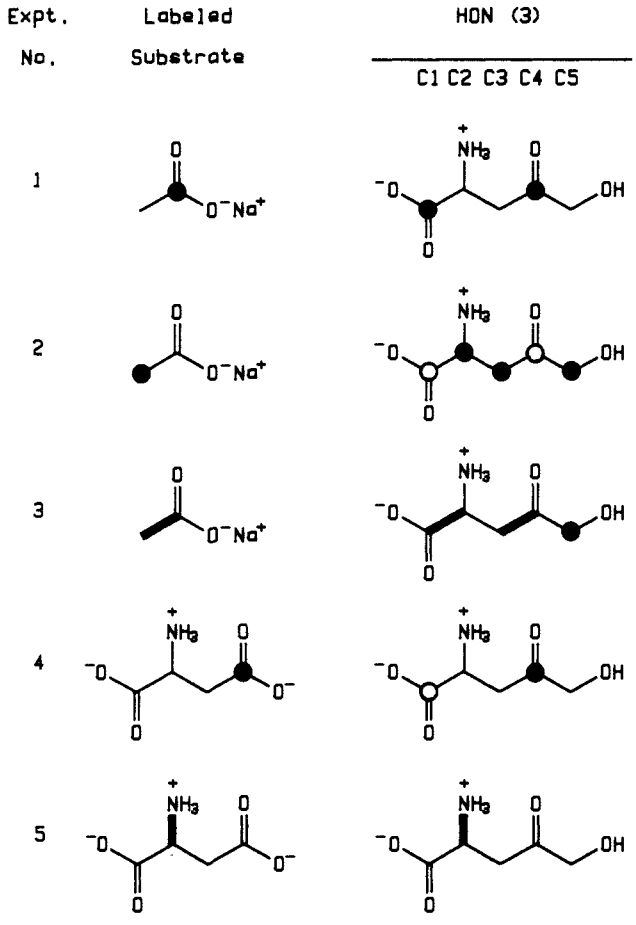


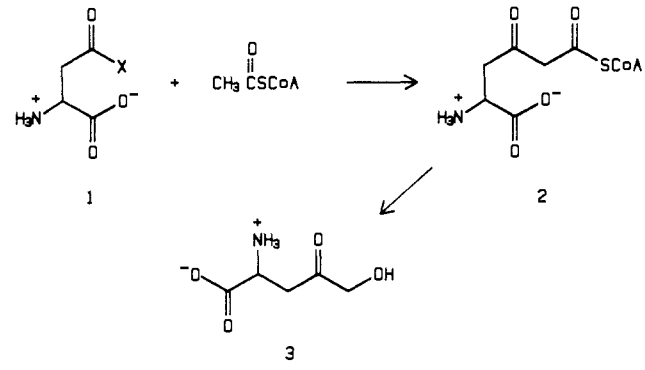
Figure 1. Observed ^{13}C enrichments and couplings in HON derived from labeled substrates. Larger enrichments (2.2-3.7 times natural abundance) are represented by filled circles, smaller enrichments (1.5-1.7 times natural abundance) are represented by open circles, and unlabeled carbon atoms correspond to natural abundance ^{13}C. Coupled nuclei are joined by heavy lines.

by these routes, would have an equal distribution of ^{13}C at C-1 and C-4.

A distinction between aspartate and oxaloacetate or malate was made by feeding DL-[2-^{13}C,^{15}N]aspartate[12] which was synthesized[13] from diethyl [2-^{13}C,^{15}N]phthalimidomalonate[14] and ethyl bromoacetate. The intact incorporation of this C-N unit, and thus the precursor role of aspartate, was demonstrated by the observation of two coupled ^{13}C NMR signals (centered at 49.1 and 48.1 ppm, $^1J_{CN}$ = ca. 6 Hz for each), in the mixture of diastereomeric γ-lactones of 4,5-dihydroxynorvaline, which corresponds to C-2 in each of the diastereomeric γ-lactones and consequently to C-2 of HON.

The pattern of incorporation of ^{13}C into HON is consistent with the condensation of acetyl coenzyme A or malonyl coenzyme A with a β-activated aspartate (**1**) to form a 6-carbon intermediate (e.g., **2**) that is converted to HON (**3**) by hydrolysis and either oxidative decarboxylation or separate decarboxylation and hydroxylation steps (Scheme I). A similar condensation between acetyl coenzyme A and a γ-activated glutamate[5] or glutamic semialdehyde[15] has been proposed as the first step in the biosynthesis of the carbapenem antibiotics. Whether the analogy

Scheme I. Biosynthetic Formation of HON (**3**) from Acetyl Coenzyme A and a β-Activated Aspartate (X = Activating Group)



extends to the enzymes that catalyze these two condensations is under investigation.

Acknowledgment. We thank the Natural Sciences and Engineering Research Council of Canada for financial support and Dr. D. L. Hooper and the Atlantic Region Magnetic Resonance Centre for providing NMR spectra. One of us (R.L.W.) is indebted to the Chemistry Department at Dalhousie University and to Dr. L. C. Vining for providing research facilities for some preliminary experiments.

Direct Measurement of Deuterium-Deuterium Dipolar Coupling and Analysis of the Ordering of a Specifically Deuteriated Diunsaturated Lipid

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Received June 28, 1988

Although polyunsaturated lipids are of considerable biological interest,[1] relatively little is known of their physico-chemical properties in membranes. In order to achieve a better understanding of their biological function we have initiated ^2H NMR studies to elucidate the average structural properties and associated molecular dynamics of liposomal polyunsaturated phospholipids.[2] As part of these studies we examined model bilayers composed of 1-palmitoyl-2-isolinoleoyl phosphatidylcholine (PiLPC) specifically deuteriated at the 8 position of the isolinoleoyl (18:2$^{\Delta 6,9}$; *cis,cis*-octadeca-6,9-dienoyl) chain (inset of Figure 1). We report here that acquiring spectra with proton decoupling facilitates the line shape analysis of the spectra and has lead to the first direct observation of geminal ^2H-^2H dipolar coupling and calculation of the complete ordering tensor for the methylene segment.

The ^2H NMR spectra of aqueous dispersions of [8'-$^2H_2$]PiLPC are relatively narrow and featureless (Figure 1a) suggesting that either the average orientation of both methylene C-^2H bonds is close to the "magic angle" (54.7°) or their molecular motion is axially asymmetric. Spectra of the sample oriented between glass plates (Figure 1c) did not resolve the uncertainty; however, acquiring spectra of both the aligned and dispersed samples, with proton decoupling (Figures 1b and 1d), established that the two deuterons are magnetically inequivalent and that their molecular

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