tin)bromochloromethane, however, attack of iodide occurred at carbon (eq 2). The product, obtained in

$$(CH_3)_3SnCBrClSn(CH_3)_3 + Na^+I^- \xrightarrow{DME} (CH_3)_3SnCIClSn(CH_3)_3 + NaBr\downarrow \quad (2)$$

63% yield, was unstable in air, turning bright orange-red on exposed surfaces. Its combustion analysis, mass spectroscopic molecular weight, and infrared spectrum were in agreement with the structure shown.

We recognize that our discovery of the first case of dihalocarbene insertion into a metal–metal bond to give a stable $-M-CX_2-M-$ system opens up a broad new field of research in the organometallic aspects of carbene chemistry. The study of compounds containing covalent metal–metal bonds has received much attention in the past few years, as the many papers on this subject show.[6] We currently are investigating reactions of phenyl(trihalomethyl)mercurials with compounds containing main group metal–main group metal bonds, main group metal–transition metal bonds, and transition metal–transition metal bonds with the aim of preparing and studying new $M-CX_2-M$ systems.

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(7) Alfred P. Sloan Foundation Fellow, 1962–1966.

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The Presence of Dehydroalanine in the Antibiotic Nisin and Its Relationship to Activity

Sir:

When the peptide antibiotic nisin[1,2] was treated with cyanogen bromide[3] (0.1 *N* HCl, 37°, 24 hr) in order to cleave the methionyl peptide bonds, a product of low molecular weight (fractions 91–110; 22 ml) was isolated by gel chromatography on a Sephadex G-25 column (6 × 120 cm; 0.2 *N* CH_3COOH).

The same product was isolated from control experiments in the absence of the reagent, indicating a bond which is labile under mildly acidic conditions. Aliquots of the pooled and lyophilized fractions from both experiments were analyzed directly using the accelerated system[4] of an amino acid analyzer.[5] A single substance was eluted from the 0.9 × 60 cm column at an effluent volume of 172–182 ml. Lysine was the only amino acid found in the total hydrolysate. The hydrolysate of the dinitrophenylated product contained only N^ϵ-dinitrophenyllysine, thus indicating the presence of an N^α-substituted derivative of lysine.

The product of the reaction of this lysine derivative with ninhydrin showed optical densities at 570 and 440 mμ which were reminiscent of those of free lysine. We therefore concluded that the N^α substituent is labile under the conditions of the ninhydrin reaction.

Similar observations had been made earlier with pyruvylamino acids formed during cleavage of the aminoacyl bond of N-aminoacyl-S-alkylcysteine peptides.[6]

The isolated product was treated with *o*-phenylenediamine.[7] Lysine was liberated in a yield of 50% after 4 hr of reaction at 37°. The fragment thus appeared to be pyruvyllysine. Since dehydroalanine peptides are cleaved with the formation of pyruvyl peptides it is implied that the COOH-terminal sequence[8] of nisin is *dehydroalanyllysine*. The release of pyruvyllysine in dilute acid is, however, slow (2%/24 hr; *cf.* below, conditions for the quantitative cleavage).

The molecular weight of nisin was determined by the method of partial substitution.[9] Monodinitrophenylnisin was isolated and purified[10] by countercurrent distribution in the system butan-1-ol–acetic acid–water, 4:3:1.

A molecular weight value of 3510 was calculated for this derivative, which is one-half that reported earlier.[2] It is, however, in agreement with the minimum molecular weight determined from the amino acid analyses of nisin (micromoles/0.5 mg; 92% recovery without dry weight correction) and *monodinitrophenylnisin* (micromoles/0.5 mg; quantitative recovery after desiccation): lysine (0.382; *0.267*), histidine (0.255; *0.265*), ammonia (0.425; *0.459*), aspartic acid (0.139; *0.144*), serine (0.119; *0.113*), lanthionine + β-methyllanthionine (0.740; *0.760*), proline (0.130; *0.129*), glycine (0.400; *0.421*), alanine (0.260; *0.270*), valine (0.134; *0.138*), methionine (0.253; *0.261*), isoleucine (0.376; *0.393*), leucine (0.257; *0.268*); mol wt (nisin), 3290; mol wt (mono-DNP-nisin), 3460.

These data indicate clearly that only one residue of lysine has been dinitrophenylated. Dinitrophenylation did not take place at the COOH-terminal lysine, since pyruvyllysine is still released from mono-DNP-nisin.

The above partial structure of the antibiotic is supported by: (a) the addition of mercaptoacetamide to the double bond of dehydroalanine at pH 4.5 and room temperature (with 1.6 m*M* nisin solution, the following values were determined for carboxymethylcysteine in the addition product: 0.24 residue, 24 hr, 1.6 m*M* mercaptan; 1.2 residues, 24 hr, 28 m*M* mercaptan; 1.2 residues, 72 hr, 56 m*M* mercaptan; the test for free sulhydryl groups with maleimide was negative and the hydrolysate was free of cystine); (b) a comparison

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(7) H. B. T. Dixon and V. Moret, *Biochem. J.*, 94, 463 (1965).

(8) Contrary to earlier reports (*cf.* ref 2) on the absence of free end groups, nisin also contains a free terminal amino group, namely, that of isoleucine.

(9) A. R. Battersby and L. C. Craig, *J. Am. Chem. Soc.*, 74, 4023 (1952).

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of the isolated nisin fragment with an authentic sample of pyruvyllysine which was synthesized according to the procedure of Bergmann and Grafe.[11] Pyruvic acid and acetamide were combined to give α,α-diacetaminopropionic acid which was converted to the azlactone by treatment with acetic anhydride on a steam bath and coupled with N^{ϵ}-carbobenzoxylysine benzyl ester. The protecting groups were removed by catalytic hydrogenation. α,α-Diacetaminopropionyllysine, mp 145° (uncor) (*Anal.* Calcd for $C_{13}H_{24}O_5N_4 \cdot H_2O$: C, 46,49; H, 7.84; N, 16.76. Found: C, 47.15; H, 7.96; N, 16.59), was treated with HCl in glacial acetic acid (110°, 10 min) to form pyruvyllysine. The synthetic product was eluted from the 60-cm column of the amino acid analyzer[4,5] at the same position as the nisin fragment. The conversion of α,α-diacetaminopropionyllysine to pyruvyllysine is quantitative, as judged by the disappearance of the peak of α,α-diacetaminopropionyllysine (effluent volume: 111–123 ml) and the appearance of only the peak corresponding to pyruvyllysine. Free lysine was absent. The ratio of the calibration value of lysine to that of pyruvyllysine is 1.56. Treatment of 1 μmole of nisin with HCl in glacial acetic acid (110°, 10 min) released 0.9 μmole of pyruvyllysine.

The antibiotic activity of nisin and its derivatives was tested against *Staphylococcus aureus* (ATCC-10537). Nisin strongly inhibits the growth of the bacterium. Monodinitrophenylnisin was also a growth inhibitor.

The addition product of nisin and mercaptoacetamide showed a very weak growth inhibitory effect, which is perhaps due to partial reactivation of the originally inactive carboxamidomethylthiolnisin. Enzyme systems capable of catalyzing this type of elimination have been described.[12]

The reaction products of the acid-catalyzed cleavage of the C^{α}–N bond of dehydroalanine in nisin, namely des-(dehydroalanyllysine)-nisin and pyruvyllysine, are both inactive against *Staphylococcus aureus*. However, when pyruvyllysine was combined with des-(dehydroalanyllysine)-nisin in a ratio of 2:3 and kept in a moist state for 48 hr at room temperature, a recombination product was obtained which again displayed antibiotic activity against *Staphylococcus aureus*. A quantitative determination showed a decrease of 70% in the original amount of pyruvyllysine. This reaction may represent a step in the biosynthesis of the antibiotic. It will undoubtedly be of importance in the contemplated synthesis of biologically active analogs of nisin.

It has thus been clearly shown for the first time that dehydroalanine is present in a naturally occurring peptide antibiotic. The biological activity of nisin is directly related to the presence of dehydroalanine in the molecule. We believe that the addition of mercaptans is the *in vitro* model reaction for the biological action of nisin. Metabolically important compounds, such as sulfhydryl-containing enzymes, glutathione, or coenzyme A, may be intercepted by nisin.

This supposition is being tested, as far as coenzyme A is concerned, on malarial parasites. These are known to be sensitive to deficiency in coenzyme A, whether this is caused by dietary host deprivation[13] or the presence of antipantothenates.[14]

Two groups of five mice each received four consecutive daily doses of (a) 500 mg/kg of nisin orally; (b) 250 mg/kg of nisin intraperitoneally. On day 4, parasite growth in the mice of group b was reduced by 98% of that on control animals. On day 7, the reduction in parasite growth was 80% for group a.

It remains to be seen whether these effects can be reversed by infusion of acetyl coenzyme A.

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(12) M. Flavin and C. Slaughter, *Biochemistry*, **3**, 885 (1964).
(13) S. Brackett, E. Woletzky, and M. Baker, *J. Parasitol.*, **32**, 435 (1945).
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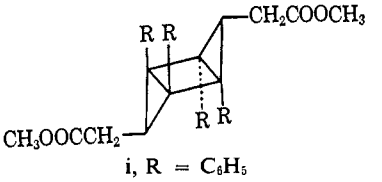
The Tricyclo[2.1.0.0^{2,5}]pentane System

Sir:

Recently it was reported that the photolysis of diazoketones **1a** and **b** in tetrahydrofuran provided ketones **2a** and **b**, respectively, and the tricyclo[2.1.0.0^{2,5}]pentane skeleton was assigned to these compounds mainly on the basis of spectral evidence.[1-3] Doering and Pomerantz, interpreting the spectral data of **2a** in a different manner, suggested an alternative structure (**3**).[4] Although the evidence then available to us and subsequent works[5] have convinced us that the tricyclic structure is the correct representation of **2a** and **b**, we have undertaken an X-ray crystal analysis of a derivative of **2a**. The result now confirms the correctness of our structure and, further, provides the precise geometry of the ring system, which is essential for understanding its unusual properties.

(1) S. Masamune, *J. Am. Chem. Soc.*, **86**, 735 (1964).
(2) Our original nomenclature is corrected: J. D. Connolly and K. H. Overton, *Ann. Rept. Progr. Chem.* (Chem. Soc. London), 348 (1964); J. Meinwald and J. K. Crandall, *J. Am. Chem. Soc.*, 88, 1292 (1966).
(3) Irradiation of a methanolic solution of **1** gave in addition to **2** (10–15%) the methyl ester of the homologated acid (20%) and a dimeric compound (20–30%), mp 236–237°, for which structure i accommodates all experimental data: H. H. Stechl, *Ber.*, **97**, 2681



i, R = C_6H_5

(1964); N. Obata and I. Moritani, *Bull. Chem. Soc. Japan*, **39**, 2250 (1966). Somewhat to our surprise, a highly purified sample of **1** evolved nitrogen very slowly upon irradiation and provided no ketone **2**. Addition of a sensitizer reproduced the products mentioned above. Copper-catalyzed reaction of **1** in refluxing benzene afforded a trace amount of **2** (at most 1%) (see ref 4).
(4) W. von E. Doering and M. Pomerantz, *Tetrahedron Letters*, 961 (1964).
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