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.

Acknowledgment. We gratefully record that the support of Dr. B. Witkop has decisively furthered this work. It is a pleasure to acknowledge the skillful assistance of Miss Patricia Q. Lee. We also wish to express our thanks to Dr. R. Schmitt for the bacterial activity tests and to Dr. G. M. Jeffery and his associates, who tested the antimalarial properties of nisin.

(11) M. Bergmann and K. Grafe, *Z. Physiol. Chem.*, 187, 187 (1930).
(12) M. Flavin and C. Slaughter, *Biochemistry*, 3, 885 (1964).
(13) S. Brackett, E. Woletzky, and M. Baker, *J. Parasitol.*, 32, 435 (1945).
(14) W. Trager, *Trans. N. Y. Acad. Sci.*, 28, 1094 (1966).

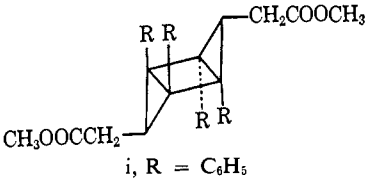
Erhard Gross, John L. Morell
National Institute of Arthritis and Metabolic Diseases
National Institutes of Health, Bethesda, Maryland 20014
Received March 18, 1967

The Tricyclo[2.1.0.0^{2,5}]pentane System

Sir:

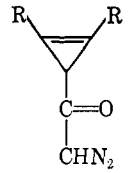
Recently it was reported that the photolysis of diazoketones **1a** and **b** in tetrahydrofuran provided ketones **2a** and **b**, respectively, and the tricyclo[2.1.0.0^{2,5}]pentane skeleton was assigned to these compounds mainly on the basis of spectral evidence.[1-3] Doering and Pomerantz, interpreting 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

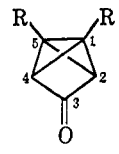


i, R = C_6H_5

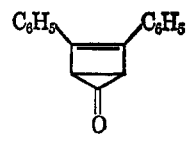
(1964); N. Obata and I. Moritani, *Bull. Chem. Soc. Japan*, 39, 2250 (1966). Somewhat to our surprise, a highly purified sample of 1 evolved nitrogen very slowly upon irradiation and provided no ketone 2. Addition of a sensitizer reproduced the products mentioned above. Copper-catalyzed reaction of 1 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).
(5) (a) S. Masamune, *ibid.*, 945 (1965); (b) S. Masamune, K. Fukumoto, Y. Yasunari, and D. Darwish, *ibid.*, 193 (1966).



1a, R = C_6H_5
b, R = n-C_3H_7



2a, R = C_6H_5
b, R = n-C_3H_7



3

Crystals of 1,5-diphenyltricyclo[2.1.0.0^{2,5}]pent-3-yl *p*-bromobenzoate, mp 138.5–139°, are triclinic: a = 5.92, b = 8.98, c = 17.85 A, α = 89° 17′, β = 82° 46′, γ = 89° 50′, Z = 2, space group $P\bar{1}$. The structure was determined with visual Cu Kα data from three-dimensional Patterson and electron-density distributions and refined by ten cycles of block-diagonal least-squares, the final R value being 0.16 for 1228 reflections. Sections of the electron-density distribution, and a corresponding diagram of the ring system, are shown in Figure 1, and the dimensions of the tricyclopentane ring system are given in Table I.

Table I. Bond Distances (Angstroms) and Valency Angles (Degrees) in the Tricyclopentane System[a]

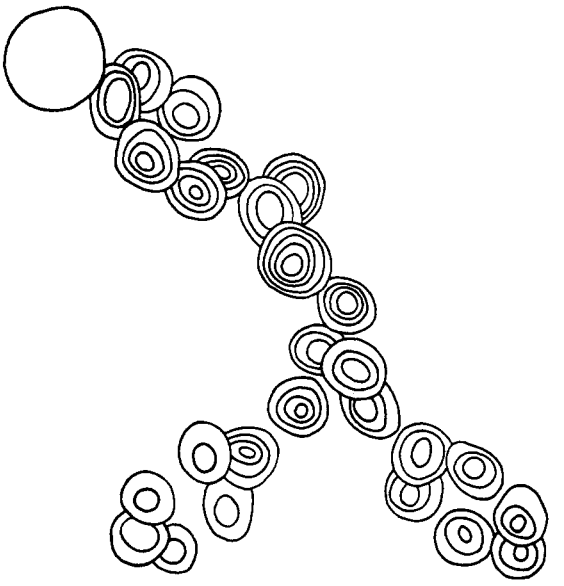
C(2)–C(3)	1.50	C(2)–C(3)–C(4)	81.7
C(3)–C(4)	1.54	C(3)–C(4)–C(5)	89.3
C(2)–C(5)	1.53	C(3)–C(4)–C(1)	92.2
C(1)–C(2)	1.54	C(3)–C(2)–C(5)	90.8
C(4)–C(5)	1.53	C(3)–C(2)–C(1)	92.9
C(1)–C(4)	1.52	C(4)–C(5)–C(2)	81.3
Mean	1.53	C(4)–C(1)–C(2)	81.4
		C(5)–C(4)–C(1)	56.3
C(1)–C(5)	1.44	C(5)–C(2)–C(1)	56.1
		C(4)–C(5)–C(1)	61.4
C(2)···C(4)	1.99	C(4)–C(1)–C(5)	62.3
		C(2)–C(5)–C(1)	62.3
		C(2)–C(1)–C(5)	61.6
External angles at C(1) and C(5) = 134.4–142.1 (six angles), mean 138		C(2)–C(3)–O	115.6
		C(4)–C(3)–O	109.5

[a] Standard deviations are 0.05 A and 3°.

The crystal analysis has confirmed the formulation of the compound as a derivative of tricyclo[2.1.0.0^{2,5}]-pentane. The great strain in the ring system is indicated by the valency angles given in Table I, there being six C–C–C angles of about 60°, three of about 80°, and four of about 90°. The C(2)···C(4) nonbonded distance is only 1.99 A. The bond distances, however, do not appear to be greatly influenced by the strain. Six of the C–C distances are in the range 1.50–1.54 A, mean value 1.53 A (standard deviation of the mean = 0.02 A), close to the normal single bond length. The bond which is common to the two three-membered rings, C(1)–C(5), measures 1.44 ± 0.05 A, so that this bond does seem to be shortened slightly, although the difference from the other bonds (0.09 A = 1.8σ) cannot be claimed to be definitely statistically significant.

All the dimensions of the substituent groups are normal. The orientations of the two phenyl groups with respect to the tricyclopentane ring system are similar, but not quite identical, and are probably influenced by intermolecular interactions.

The geometry of a derivative of **2** now being known, we understand better the unusual spectral behavior of this strained system. It is interesting to note that mass spectra of **2** and the corresponding hydroxy compound exhibited, in addition to their parent peaks, pronounced peaks at M − CO[4] and M − CHO, respectively. The interpretation of these peaks, of course, must await further studies.[6]



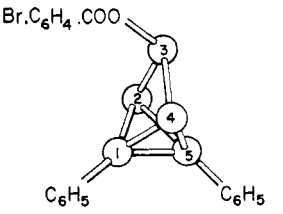


Figure 1.

(6) We thank the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research.

James Trotter, C. S. Gibbons
Department of Chemistry, University of British Columbia
Vancouver 8, British Columbia, Canada

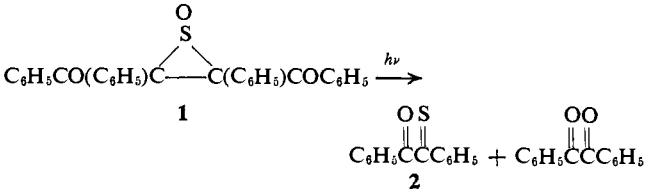
Nobuo Nakatsuka, S. Masamune
Department of Chemistry, University of Alberta
Edmonton, Alberta, Canada

Received March 20, 1967

Photolysis of Dibenzoylstilbene Episulfoxide. Formation of Monothiobenzil[1]

Sir:

Irradiation of dibenzoylstilbene episulfoxide[2] (**1**) in benzene at 7–11° gives deep blue monothiobenzil[3]



(1) This work was supported in part by Grant GP-5513 of the National Science Foundation.

(2) D. C. Dittmer and G. C. Levy, *J. Org. Chem.*, 30, 636 (1965). Its stereochemistry is unknown.

(3) No previous characterization of monothiobenzil has been reported, although it has been suggested as a decomposition product of