yield to a 15,16-di-*O*-acetyl derivative with the remaining 50% present as a mixture of mono-*O*-acetyl derivatives.

Trimethylsilylation of alkaloids was conducted in pyridine with bis(trimethylsilyl)fluoroacetamide for 1 h at room temperature. Under these conditions, V was converted in only 30% yield to an *O*-trimethylsilyl derivative, while the dihydro reduction product was converted in almost quantitative yield to an *O*-trimethylsilyl derivative.

All reactions were carried out with 10–100 μg of alkaloid.

Crystals of V were prepared as follows: The free base (11 mg) was converted to the hydrochloride salt with a stoichiometric amount of methanolic HCl. After concentration in vacuo, it was crystallized at room temperature from ethyl acetate–cyclohexane.

Acknowledgments. The authors express their gratitude to Dr. Peter Roller, National Cancer Institute, Bethesda, Md., VG-Organic Ltd. and AEI Scientific Instruments Ltd., Manchester, England, for high-resolution mass spectral-gas chromatographic analyses of alkaloids from *Dendrobates tricolor*, to G. McDonald and J. Mermelstein for the 360-MHz spectra, and to Dr. Charles W. Myers of the American Museum of Natural History without whose collaboration the investigation of dendrobatid frogs would be impossible.

Supplementary Material Available: Listings of observed and calculated structure factors as well as tables of anisotropic thermal parameters for the nonhydrogen atoms and coordinates for hydrogen atoms (8 pages). Ordering information is given on any current masthead page.

References and Notes

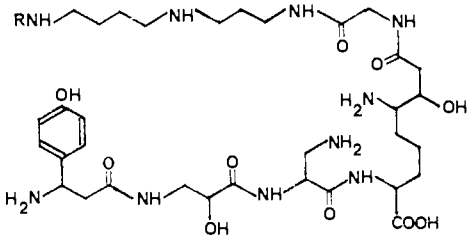
(1) (a) Laboratory of Bioorganic Chemistry, National Institute of Arthritis Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Md. (b) Faculty of Science, Osaka City University, Sugimotocho, Syuiyoski-Ku, Osaka, Japan. (c) Laboratory of Chemistry, National Heart, Lung, and Blood Institute, National Institutes of Health. (d) Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, D.C. 20390.
(2) J. W. Daly, G. B. Brown, M. Mensah-Dwumah, and C. W. Myers, *Toxicon*, **16**, 163 (1978).
(3) E. X. Albuquerque and J. W. Daly, "The Specificity and Action of Animal, Bacterial and Plant Toxins: Receptors and Recognition, Ser. B", Vol. 1, P. Cuatrecasas, Ed., Chapman and Hall, London, 1977, p 297.
(4) (a) J. W. Daly and C. W. Myers, *Science*, **156**, 970 (1967); (b) M. Mensah-Dwumah and J. W. Daly, *Toxicon*, **16**, 189 (1978); (c) M. A. Maleque, E. X. Albuquerque, J. E. Warnick, J. W. Daly, and Y. Nimitkitpaisan, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **38**, 1399 (1979).
(5) A. J. Lapa, E. X. Albuquerque, J. M. Sarvey, J. Daly, and B. Witkop, *Exp. Neurol.*, **47**, 558 (1975); M. E. Eldefrawi, A. T. Eldefrawi, N. A. Mansour, J. W. Daly, B. Witkop, and E. X. Albuquerque, *Biochemistry*, **17**, 5474 (1978).
(6) T. Tokuyama, J. Daly, B. Witkop, I. L. Karle, and J. Karle, *J. Am. Chem. Soc.*, **90**, 1917 (1968); T. Tokuyama, J. Daly, and B. Witkop, *ibid.*, **91**, 3931 (1969).
(7) J. W. Daly, T. Tokuyama, G. Habermehl, I. L. Karle, and B. Witkop, *Justus Liebigs Ann. Chem.*, **729**, 198 (1969).
(8) J. W. Daly, B. Witkop, T. Tokuyama, T. Nishikawa, and I. L. Karle, *Helv. Chim. Acta*, **60**, 1128 (1977).
(9) J. W. Daly, I. Karle, C. W. Myers, T. Tokuyama, J. A. Waters, and B. Witkop, *Proc. Natl. Acad. Sci. U.S.A.*, **68**, 1870 (1971); T. Tokuyama, K. Yenoyama, G. Brown, J. W. Daly, and B. Witkop, *Helv. Chim. Acta*, **57**, 2597 (1974).
(10) The nearly 100 dendrobatid alkaloids reported in ref 2 were assigned numerical designations based on molecular weight and, in the case of more than one alkaloid with the same molecular weight, by a letter (A, B, C, etc.). For example, the alkaloid (V) isolated from *D. tricolor* and characterized by X-ray crystallography in the present paper was designated *251D* based on its detection in a number of other species of dendrobatid frogs. The designations from ref 2 are cited as appropriate throughout the present paper.
(11) J. Karle, *Acta Crystallogr., Sect. B*, **24**, 182 (1968).
(12) I. L. Karle, H. Hauptman, J. Karle, and A. G. Wing, *Acta Crystallogr.*, **11**, 257 (1958).
(13) J. M. Bijvoet, A. F. Peerdeman, and A. J. van Bommel, *Nature (London)* **168**, 271 (1951).
(14) W. C. Hamilton, *Acta Crystallogr.*, **18**, 502 (1965).
(15) C. Altona, H. J. Geise, and C. Romers, *Tetrahedron*, **24**, 13 (1968).
(16) See paragraph at end of paper regarding supplementary material.
(17) Voucher specimens of *Dendrobates tricolor* and *Dendrobates pumilio* are in the collections of the American Museum of Natural History, New York.
(18) This compound will be designated *217* according to the numbering system for dendrobatid alkaloids introduced in ref 2 (see footnote 10).

Communications to the Editor

Biosynthesis of Amino Acids. Investigation of the Mechanism of β-Tyrosine Formation

Sir:

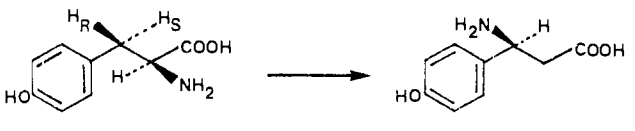
Cultures of *Bacillus brevis* Vm4 produce two peptide antibiotics, edeine A and edeine B (**1** and **2**), that contain a group



1, R = H

2, R = $H_2N\overset{|}{C}=NH$

of novel amino acids.[1] One of these amino acids is β-tyrosine (**3**), which is formed by the isomerization of L-α-tyrosine (**4**)



4 3

catalyzed by the enzyme tyrosine α,β-mutase.[2] The mutase enzyme is unusual in that it requires ATP, is inhibited by reagents reacting with carbonyl groups, and has no requirement for pyridoxal phosphate. To provide additional information concerning the mechanism of the mutase reaction, we have examined the conversion of doubly labeled forms of α-tyrosine into β-tyrosine.

3(*R*,*S*)-[3-^{3}H,3-^{14}C]-DL-α-Tyrosine[3] was administered to cultures of *B. brevis* Vm4 and the β-tyrosine produced was isolated and purified by paper chromatography. Further purification was accomplished by dilution and repeated recrystallization to constant specific activity and constant $^3H/^{14}C$ ratio. The results of this experiment are outlined in Table I (expt 1). The $^3H/^{14}C$ ratio of the purified β-tyrosine clearly shows that amino group migration is accompanied by the stereospecific removal of one hydrogen atom from C-3 of α-tyrosine (expected loss is 50%). Additional experiments were carried out to define the stereospecificty of this hydrogen loss. Samples of (3*R*)-[3-^{3}H]- and (3*S*)-[3-^{3}H]-α-DL-tyrosine were prepared,[4] mixed with [3-^{14}C]-DL-α-tyrosine, and administered to *B. brevis* cultures. Interpretable results were not obtained in these in vivo experiments, presumably because of metabolism of the α- or β-tyrosine via pathways unrelated to the migration process. The samples of chirally labeled α-tyrosine were therefore converted into β-tyrosine in vitro, using the purified mutase enzyme.[2] The β-tyrosine isolated in these experiments gave the $^3H/^{14}C$ ratios shown (Table I, expt 2, 3). The data indicate clearly that the migration of the amino

0002-7863/80/1502-0836$01.00/0

Table I. Conversion of Doubly Labeled α-Tyrosine into β-Tyrosine

expt.	precursor ($^3H/^{14}C$ ratio)	$^3H/^{14}C$ for β-tyrosine	% ^3H retention
1[a]	3(*R,S*)-[3-^{3}H,3-^{14}C]-DL-α-tyrosine (8.56)	4.56	53
2[b]	3(*S*)-[3-^{3}H,3-^{14}C]-DL-α-tyrosine (5.87)	1.35	23
3[b]	3(*R*)-[3-^{3}H,3-^{14}C]-DL-α-tyrosine (2.25)	1.86	83
4[b]	[2-^{3}H,1-^{14}C]-L-α-tyrosine (4.43)	0.71	16

[a] In vivo experiment. [b] In vitro experiment.

group from C-2 of α-tyrosine to C-3 leads to loss of the 3-*pro-S* hydrogen atom of the amino acid. The lack of complete tritium loss or retention observed in these two experiments is probably due to the fact that the chirally tritiated forms of α-tyrosine are only ~85% optically pure.[4]

A complete picture of the stereospecificity of the amino group migration to C-3 requires that the absolute configuration of natural β-tyrosine be known. The absolute configuration was determined in the following way. Resolution of 3-formamido-3-(*p*-methoxyphenyl)propionic acid with (+)-cinchonine gave (*R*)-(+)-3-formamido-3-(*p*-methoxyphenyl)-propionic acid with $[\alpha]_D$ +128° (93% optically pure).[5,6] Demethylation and deformylation of the optically active formamido acid with refluxing hydrobromic acid yielded β-tyrosine which was purified by recrystallization and then converted into the hydrochloride salt. Recrystallization of the hydrochloride gave (*R*)-β-tyrosine hydrochloride which exhibited an $[\alpha]_D$ −4.15°. Since β-tyrosine hydrochloride isolated from edeine A and B exhibits an $[\alpha]_D$ +7.8°, it follows that naturally occurring β-tyrosine has the *S* configuration.[7] This information combined with the knowledge that the 3-*pro-S* hydrogen atom of α-tyrosine is lost during amino group migration leads to the conclusion that the amino group is introduced at C-3 with inversion of configuration.

The loss of the 3-*pro-S* hydrogen atom from α-tyrosine as the result of β-tyrosine formation suggested that the mechanism of the α-tyrosine mutase reaction might be related to that of the reactions catalyzed by the ammonia-lyase enzymes.[8] All of the ammonia-lyases that have been examined catalyze a loss of ammonia that involves the removal of the same hydrogen atom in an absolute sense as that removed from C-3 of α-tyrosine by the mutase enzyme. In addition, both histidine and phenylalanine ammonia-lyase are deactivated by reagents that react with carbonyl groups. Evidence has been obtained that indicates that the electrophilic group present at the active sites of these ammonia-lyases is a dehydroalanyl residue. At present, little is known regarding the nature of the electrophilic group that occurs at the active site of α-tyrosine mutase. To gain some insight into the possible nature of the electrophilic group in tyrosine mutase, an in vitro experiment was carried out with [2-^{3}H]-L-α-tyrosine.[9] The results of this experiment are shown in Table I (expt 4): the in vitro conversion of [1-^{14}C,2-^{3}H]-L-α-tyrosine into β-tyrosine proceeds with loss of most of the tritium label from C-2 of α-tyrosine.[10] This observation is compatible with the formation of a Schiff base between a carbonyl group at the active site of the mutase and the amino group of α-tyrosine. It is apparently not consistent with the formation of an adduct between the amino group of α-tyrosine and a dehydroalanyl residue since the ammonia-lyase enzymes do not catalyze exchange of the α-hydrogen atoms of their amino acid substrates. The results of expt 4 also serve to emphasize the differences between the α-tyrosine mutase enzyme and an L-lysine 2,3-aminomutase found in *Clostridium*.[11] The lysine mutase requires pyridoxal phosphate and *S*-adenosylmethionine as cofactors and it catalyzes an isomerization that proceeds without incorporation of hydrogen from the medium. The stereospecificity of the lysine mutase reaction remains to be determined.

References and Notes

(1) (a) Roncari, G.; Kurylo-Borowska, Z.; Craig, L. C. *Biochemistry* **1966**, *5*, 2153. (b) Hettinger, T. P.; Craig, L. C. *Ibid.* **1968**, *7*, 4147. (c) Hettinger, T. P.; Kurylo-Borowska, Z.; Craig, L. C. *Ibid.* **1968**, *7*, 4153.
(2) Kurylo-Borowska, Z.; Abramsky, T. *Biochim. Biophys. Acta* **1972**, *264*, 1.
(3) Parry, R. J. *J. Chem. Soc., Chem. Commun.* **1975**, 144.
(4) Kirby, G. W.; Michael, J. *J. Chem. Soc., Perkin Trans. 1* **1973**, 115. Strange, P. G.; Staunton, J.; Wiltshire, H. R.; Battersby, A. R.; Hanson, K. R.; Havir, E. A. *Ibid.* **1972**, 2364.
(5) Keller-Schierlein, W.; Klaus, A.; Naegeli, H. U.; Wolf, G.; Zähner, H. *Experientia* **1975**, *31*, 1001.
(6) Resolution using (+)-cinchonine was found to be more efficient than resolution using (−)-cinchonidine as described in ref 5.
(7) Assuming that the rotation reported for natural β-tyrosine hydrochloride corresponds to 100% optical purity, then the synthetic (*R*)-β-tyrosine hydrochloride has an optical purity of ~53%. Apparently some racemization of the asymmetric center occurs during the hydrobromic acid treatment.
(8) Havir, E.; Hanson, K. "The Enzymes", Boyer, P., Ed.; Academic Press: New York, 1973; Vol. 7, p 75.
(9) Brundish, D. E.; Elliott, D. F.; Wade, R. *J. Labelled Compd.* **1971**, *7*, 473.
(10) Loss of label due to the presence of a tyrosine racemase can be ruled out since the purified enzyme does not utilize D-α-tyrosine as a substrate. A referee has suggested that the tritium loss might be due to the presence of a tyrosine transaminase in the enzyme preparation. The failure[2] to detect pyridoxal phosphate in the enzyme preparation makes this explanation seem unlikely.
(11) Chirpich, T. P.; Zappia, V.; Costilow, R. N.; Barker, H. A. *J. Biol. Chem.* **1970**, *245*, 1778.
(12) Recipient of a Public Health Service Research Career Development Award (7 K04 GM00472) from the National Institute of General Medical Sciences, 1975–1980.

Ronald J. Parry*[12]
Department of Chemistry, Rice University
Houston, Texas 77001

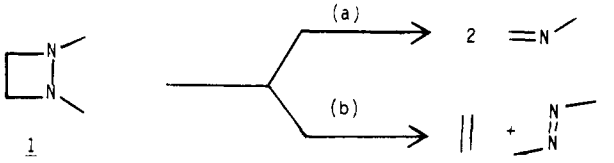
Z. Kurylo-Borowska
Department of Biochemical Genetics
The Rockefeller University, New York, New York 10021

Received July 23, 1979

Flash Vacuum Thermolysis of 1,2-Diazetidines

Sir:

Thermolysis of unsaturated six-membered-ring hydrazines, 1,2,3,6-tetrahydropyridazine derivatives, generates dienes and azo compounds,[1] and the synthetic utility of this cycloreversion as a source of diimide has been well established.[1d] Nelsen[1a] has proposed that this cycloreversion is a concerted retro-Diels–Alder reaction. However, analogous thermolytic studies on four-membered-ring hydrazines, 1,2-diazetidines, are few.[2] For unsubstituted 1,2-diazetidines **1**,



two cycloreversions are conceivable, namely, (a) cleavage to imines and (b) fragmentation to an alkene and an azo compound. We have prepared the fused-ring 1,2-diazetidine[3] **2** for which four unique thermolytic pathways are possible: (1) a $[2\pi + 2\sigma + 2\sigma]$ reversion to quadricyclane and azomethane; (2) a $[2\sigma + 2\sigma]$ reversion to norbornadiene and azomethane; (3) a $[2\pi + 2\sigma + 2\sigma]$ reversion to cyclopentadiene and Δ^3-1,2-dimethyl-1,2-diazetine (**3**); and (4) a $[2\sigma + 2\sigma]$ cycloreversion to diimine **4**.

0002-7863/80/1502-0837$01.00/0