what extent the use of more complete molecular wave functions would alter the numerical values of J_c/J_t derived from eq 1 is unknown. Such a treatment, however, may preclude the equivalency of J_c and J_t in planar **1** (*i.e.*, when $\omega = 2\phi$). Also, indirect σ-electron contributions to five-bond allylic–allylic couplings generally may not be negligible.

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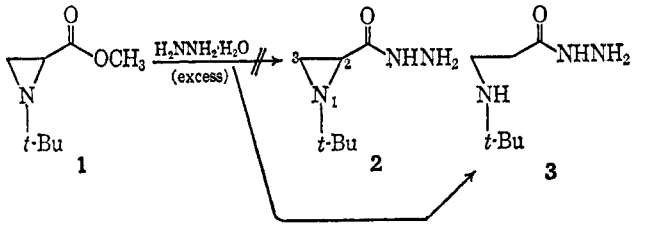
Diimide Formation *via* an Aziridine Rearrangement

Sir:

An attempt to prepare N-*t*-butylaziridine-2-carboxylic acid hydrazide (**2**) according to standard procedures[1] from ester **1**[2] and excess hydrazine hydrate at room temperature (4 days) or at reflux (5 hr in ethanol) resulted in copious gas evolution. The sole organic product of the reaction was identified as hydrazide **3**.[3] This

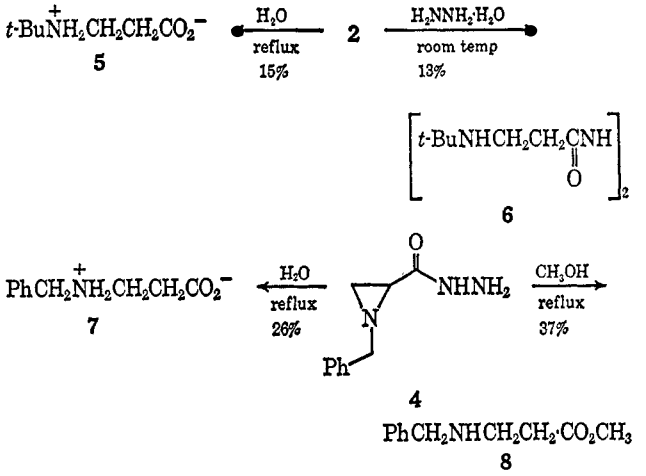


ring-opening reduction was neither expected nor with apparent precedent and thus prompted further investigation.

A solution of **1** (1.1 molar excess) and hydrazine hydrate was allowed to stand at room temperature for 9.5 hr. Analysis of the resultant mixture (in D_2O) by nmr spectroscopy revealed the formation of methanol as well as a slight change in the pattern and chemical shift of the characteristic three-proton aziridine ring multiplet. Although its instability precluded isolation, we assign structure **2**, the originally expected hydrazide, to the product. In a similar manner, the N-benzyl analog **4**[4] could be prepared and isolated as a spectrally pure, crystalline precipitate (66%). Formation and identification of products **2** and **4** thus rule out any form of direct reductive ring scission by hydrazine.[5]

Characteristic reactions of **2** and **4** are shown in Scheme I. In each case cleavage occurred at both the N_1–C and CO–N bonds. Diimide **9** and amino ketene **10** constitute possible products of such cleavage. The

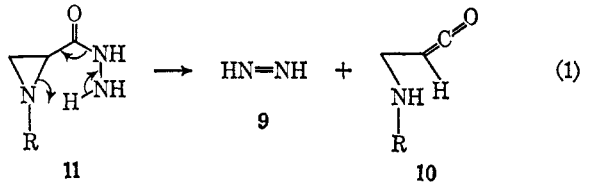
Scheme I

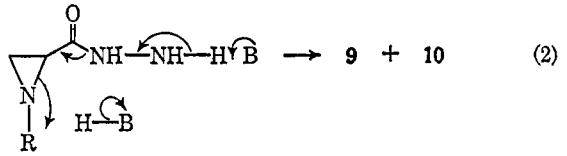


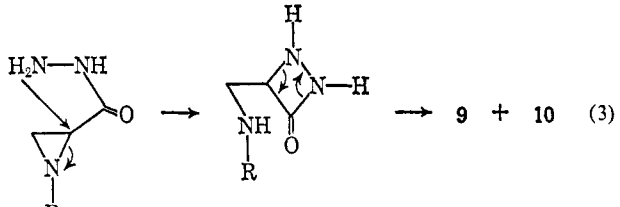
former would account for the observed gas evolution and the latter for products **3**, **5**,[3] **6**,[6] **7**,[7] and **8**.[8] Confirmatory evidence for the intermediacy of diimide was, in fact, obtained from the observation of concurrent reduction of azobenzene to hydrazobenzene in the conversion of **4** to **8**.[9] Based on the above evidence, a

4 (fourfold excess) + PhN=NPh ⟶ PhNHNHPh + **8**

number of alternative formulations for this rearrangement may be considered[10] (eq 1–3). Although we can-







not distinguish between paths 1 and 2, path 3 appears unlikely in view of the known and predicted behavior of 1,2-diazetidine-3-ones[11] as well as the known course of intramolecular nucleophilic epoxide and aziridine ring opening.

(1) R. L. Shriner, R. C. Fuson, and D. Y. Curtin, "The Systematic Identification of Organic Compounds," 5th ed, John Wiley and Sons, Inc., New York, N. Y., 1964, p 273.

(2) Prepared by a procedure similar to that of M. A. Stolberg, J. J. O'Neill, and T. Wagner-Jauregg, *J. Am. Chem. Soc.*, **75**, 5045 (1953); C. L. Moyer, unpublished results.

(3) This compound was characterized by microanalysis and by ir, nmr, and mass spectroscopy.

(4) This compound was characterized by nmr, ir, and mass spectroscopy. It was also converted to a stable acetone hydrazone.[3]

(5) In agreement with this hypothesis is the observation that representative aziridines, *e.g.*, N-*t*-butyl-2-aziridinecarbinol and 1,2-diphenylaziridine, were stable to hydrazine hydrate in methanol at room temperature.

(6) Compound **6**[3] crystallized from an attempted preparation of **2** in which equimolar amounts of **1** and hydrazine hydrate were used. It apparently is formed from the reaction of **3** with **10**.

(7) This compound was identical with an authentic sample: A Zilkha, E. S. Rachman, and J. Rivlin, *J. Org.Chem.*, **26**, 376 (1961).

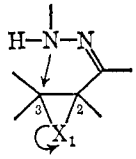
(8) The hydrochloride of **8** was identical with an authentic sample: P. L. Southwick and R. T. Crouch, *J. Am. Chem. Soc.*, **75**, 3413 (1953).

(9) The reduction was followed by tlc. Hydrazobenzene was recovered from the reaction mixture and shown to be identical with that prepared by the method of E. J. Corey, W. L. Mock, and D. J. Pasto, *Tetrahedron Letters*, 347 (1961).

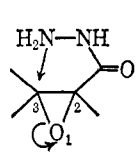
(10) These formulations are not intended to imply any necessarily concerted timing in the events leading from **11** to the products. A mechanism similar to (2) has been postulated for the formation of diimide and ketenes from the reactions of mono-, di-, and trichloroacetyl hydrazide hydrochlorides with base: R. Buyle, *Helv. Chim. Acta*, **47**, 2449 (1964).

(11) L. Horner and E. Spietschka, *Chem. Ber.*, **89**, 2765 (1956).

The reactions of **2** and **4** stand in sharp contrast to those of structurally similar heterocyclic systems (*e.g.*, **12** and **13**). In these systems rearrangement apparently



12,[12] X = O, NR



13[13]

proceeds *via* attack at C-3 with resultant formation of a five-membered ring. Electronegativity of the heteroatom and steric factors are among the variables which might be expected to influence the direction and ease of rearrangement. The scope, utility, and detailed mechanism of this and similar rearrangements are under further investigation.

Acknowledgment. Support of this research by a National Science Foundation Grant (GP-5531) and by a NASA traineeship to S. C. C. (1965–1968) is gratefully acknowledged.

(12) A. Padwa, *J. Org. Chem.*, **30**, 1274 (1965); N. H. Cromwell, N. G. Barker, R. A. Wankel, P. J. Vanderhorst, F. W. Olson, and J. H. Anglin, Jr., *J. Am. Chem. Soc.*, **73**, 1044 (1951); N. H. Cromwell and H. Hoeksema, *ibid.*, **71**, 716 (1949).

(13) V. F. Martynov and I. B. Belov, *J. Gen. Chem. USSR*, **31**, 1398 (1961). The mechanism of this reaction has not been established.

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Biosynthetic Studies. II.[1] The Mode of Incorporation of Phenylalanine into Gliotoxin

Sir:

In our recent studies on biosynthesis,[1,2] we have employed precursors enriched with stable isotopes (^{15}N, ^{13}C) and spectral methods for the determination of the site and extent of labeling. We report here our findings from a series of experiments using intermolecularly doubly labeled amino acids (see Table I) which provide some insight into the biotransformations of phenylalanine, an important precursor of many families of natural products.

It has been reported[3] that phenylalanine is incorporated efficiently into gliotoxin (I). We have found[1] that during the production of gliotoxin by *Trichoderma viride* in a chemically defined medium, if [^{15}N]glycine be added to the substrate,[4] both nitrogen atoms in I are labeled although to unequal extents.[1] The presence of [^{15}N]phenylalanine in the substrate, however, leads to the labeling of only N-5 in I. It is difficult to determine whether phenylalanine is incorporated intact into I to any appreciable extent, but the wide divergence in the isotope dilution observed (see Table I) when a mixture of [1-^{14}C]phenylalanine and [^{15}N]-

(1) Part I: A. K. Bose, K. G. Das, P. T. Funke, I. Kugajevsky, O. P. Shukla, K. S. Khanchandani, and R. J. Suhadolnik, *J. Am. Chem. Soc.*, **90**, 1038 (1968).

(2) A. K. Bose, P. T. Funke, K. G. Das, and R. J. Suhadolnik, Abstracts, 4th International Symposium on the Chemistry of Natural Products, Stockholm, June 1966, p 150; A. K. Bose, Second Natural Products Symposium, University of the West Indies, Jan 1968.

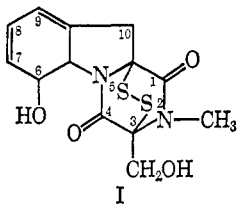
(3) J. A. Winstead and R. J. Suhadolnik, *J. Am. Chem. Soc.*, **82**, 1644 (1960).

(4) In each experiment 50 mg of the labeled compound was added to about 1.5 l. of medium. For experimental details see ref 1.

Table I

Precursor	Gliotoxin: Isotope level, %	Gliotoxin: Isotope dilution
[1-^{13}C]Phe	^{13}C, 6	8.4 ×
[1-^{14}C]Phe		8.1 ×
[1-^{13}C]Phe	^{13}C, 5	10.0 ×
[3-^{14}C]Phe		10.3 ×
[1-^{14}C]Phe	^{15}N, 9	4.4 ×
[^{15}N]Phe		11.1 ×
[^{15}N]Asp	^{15}N, 2.5	
[1-^{14}C]Asp	^{14}C, 0	
[^{15}N]Glu	^{15}N, 2.5	
[1-^{14}C]L-Phe	^{15}N, 9	4.3 ×
[^{15}N]DL-Phe		11.1 ×
[1-^{14}C]D-Phe	^{15}N, 11	2.6 ×
[^{15}N]DL-Phe		9.1 ×

phenylalanine is used indicates that extensive deamination and reamination must occur. Aspartic acid causes labeling at both nitrogen atoms in gliotoxin without contributing to the carbon skeleton. Glutamic acid also serves as the source of both nitrogen atoms in I. One has to conclude that, in the system under examination, phenylalanine and N-methylserine (or serine) readily participate in a nitrogen pool but the exchange of the amino group between phenylalanine and serine (or its equivalent) is limited.



I

The experiment with phenylalanine labeled at C-1 with ^{13}C as well as with ^{14}C established that there is no appreciable isotope effect during incorporation, as is to be expected. Therefore, the observation that [1-^{13}C]phenylalanine and [3-^{14}C]phenylalanine are incorporated with the same isotope dilution (within limits of experimental error) signifies that the carbon skeleton of phenylalanine remains intact during deamination and reamination. At present there is insufficient information for determining whether the C-6,C-3 unit is in the form of phenylpyruvic or cinnamic acids or their equivalents. However, the finding that both L- and D-phenylalanines are equally efficient precursors of gliotoxin is compatible with an optically inactive intermediate.

The significantly higher dilution of ^{15}N compared to ^{13}C or ^{14}C during incorporation is indicative of a larger nitrogen pool than carbon pool. The only source of nitrogen in the medium used is NH_4^+. Since the concentration of this ion is quite large,[4] it is remarkable that a ^{15}N-enrichment level as high as 9% was obtained.

Work is in progress for obtaining further details about the incorporation of phenylalanine and *m*-tyrosine into gliotoxin.

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