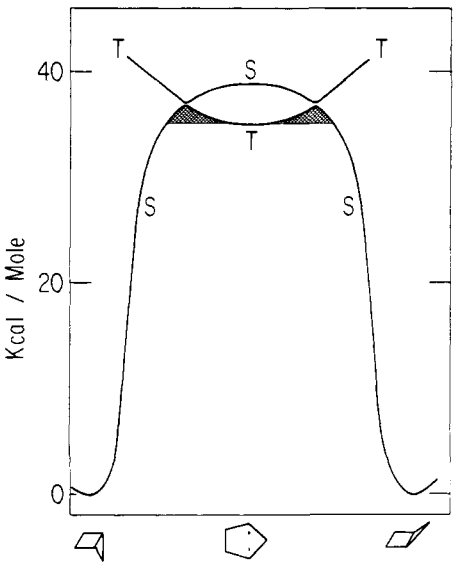

Figure 2. Schematic energy surface relating II and III. Height of singlet maximum (39 kcal/mol) is taken from J. P. Chesick, *J. Am. Chem. Soc.*, **84**, 3250 (1962). The cross hatched area is the barrier through which the system tunnels via a normal mode consisting mainly of a ring bending vibration.

ture range studied with a difference between the fast and slow component of approximately a factor of two. This behavior has precedent[10] and is presumably due to an ensemble of slightly different host sites, differing in their restraining power for the out-of-plane motion accompanying the formation of III. In addition both components of the rate are essentially temperature independent between 1.3 and 20°K! Only above 20°K does the disappearance of II begin to show a significant temperature dependence. The rate of II-d_8 at 5.5°K is too slow to study. This behavior is characteristic for quantum mechanical tunneling through a low barrier.[11] Estimates of the shape of the barrier are made difficult by the fact that the extremely small transmission coefficient (10^{-15} to 10^{-16} sec^{-1}) has a contribution from the spin forbiddenness of the reaction. Nevertheless, considering the large reduced mass of the tunneling groups, it is hard to see how the barrier height could exceed 2 kcal/mol.[12] A more detailed study of the kinetic behavior above 20°K on II and II-d_8 should yield a better estimate.

The results reported here are of interest in connection with the problem of whether the singlet surface has a minimum associated with structure II. While thermochemical calculations indicate such a well to be 13 kcal/mol deep[13] our results appear to be more consistent with a surface as indicated in Figure 2 where there is no minimum with singlet character. This conclusion is in line with quantum mechanical calculations on trimethylene.[14]

Acknowledgments. Thanks are due to Professor C. A. Hutchison, Jr., and Dr. M. D. Kemple, who made available their equipment and helped in the experiments at 1.3°K.

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The Nonparticipation of α,β-Dehydrovalinyl Intermediates in the Formation of δ-(L-α-Aminoadipyl)-L-cysteinyl-D-valine

Sir:

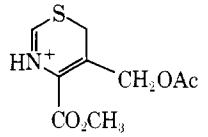
The tripeptide δ-(α-aminoadipyl)cysteinylvaline was first isolated from *Penicillium chrysogenum* in 1960,[1] but the absolute configurations of its constituent amino acids were not determined. Subsequently, a similar noncyclic peptide was isolated from *Cephalosporium acremonium*, characterized as δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine (ACV)[2] and shown to be formed from δ-(L-α-aminoadipyl)-L-cysteine and L-valine, but not D-valine, in cell extracts.[3,4] More recently, ACV was shown to be a precursor of penicillin N as manifested by its incorporation into the latter β-lactam antibiotic by a cell-free system obtained from protoplasts of *C. acremonium*.[5]

Several recent publications[6,7] report the asymmetric incorporations of chirally labeled methylvaline-*methyl*-^{13}C and methylvalines-*methyl*-d_3 into β-lactam antibiotics. We present here the results of biosynthetic studies with (2*S*,3*S*)-methylvaline-^{15}N,*3-methyl*-d_3 and experimental evidence for the nonparticipation of α,β-dehydrovalinyl intermediates in the biosynthesis of ACV.

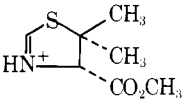
Exposure of mesaconic-*methyl*-d_3 acid[7] to β-methylaspartase[8] in the presence of $^{15}NH_3$[9] afforded L-*threo*-β-methylaspartic-^{15}N,*methyl*-d_3 acid in 53% yield, which was transformed into (2*S*,3*S*)-methylvaline-^{15}N,*3-methyl*-d_3[10] via the sequence of reactions previously described[6] in an overall yield of 23%.

After incubation of (2*S*,3*S*)-methylvaline-^{15}N,*3-methyl*-d_3 with washed cells of *C. acremonium* mutant C91[11] for 10 hr, the resulting penicillin N and cephalosporin C were isolated[6] and subjected to mass spectrometric analyses[12] as their *N*-benzoylpenicillin N methyl ester and *N*-acetylcephalosporin C methyl ester derivatives,[7] respectively. The most intense mass fragments at *m/e* 174[13] and 230, possessing the valinyl moieties of penicillin N and cephalosporin C, respectively, were selected for the calculation of isotopic ratios. The data in Table I clearly demonstrate that the entire skeleton[14] of (2*S*,3*S*)-methylvaline-^{15}N,*3-methyl*-d_3 is incorporated intact into penicillin N and cephalosporin C. This conclusion is vindicated by the very prominent (P + 4) peak at m/e 178 and the (P + 3) peak at m/e 233, respectively. It is evident that the valine molecule underwent

Table I. Isotopic Distribution of Cephalosporin C and Penicillin N Derivatives Derived from (2*S*, 3*S*)-Methylvaline-^{15}N,*3-methyl-d*$_3$



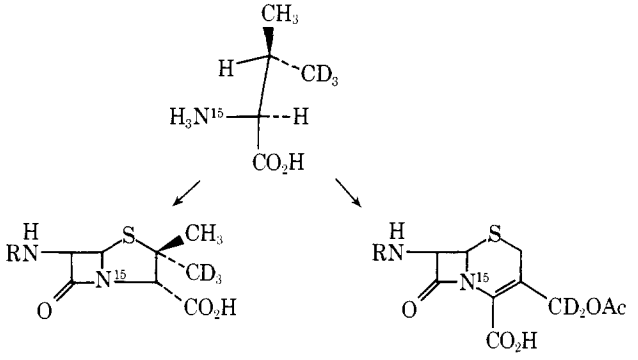
m/e 230



m/e 174

m/e	Ceph C	*m/e*	Pen N
230	63.2	174	63.4
231	6.5	175	2.0
232	24.1	176	1.6
233	6.2	177	27.6
		178	5.4

transamination to a considerable extent as indicated by the appearance of (P + 3) and (P + 1) peaks for penicillin N and (P + 2) and (P + 1) peaks for cephalosporin C. As the isotopic ratios of the valinyl moieties in both penicillin N and cephalosporin C were found to be very similar, this finding is consistent with the proposition that both β-lactam antibiotics originate from a common tripeptide intermediate such as ACV.



We next turned our attention to the question whether α,β-dehydroamino acid units are involved during the incorporation of L-valine into ACV, since an α,β-dehydrovaline derivative of a tripeptide has been proposed as a possible common intermediate in the biosynthesis of penicillin and cephalosporin antibiotics.[15-17] As cell extracts of *C. acremonium* incorporated only small quantities of L-valine-^{14}C into ACV, uniformly labeled L-valine-^{14}C (20 μCi)[18] was exposed to washed starved cells of *C. acremonium*[11] for 15 min to yield ACV-^{14}C (0.36 μCi), which was isolated as its sulfonic acid derivative.[2] After acid hydrolysis, the radioactive valine (0.19 μCi) was isolated and its absolute configuration was confirmed to be that of D configuration by use of L- and D-amino acid oxidases.[11] In a similar fashion, L-[2,3-^{3}H]valine (40 μCi)[18] was fed and the resulting tritiated ACV (0.4 μCi) was isolated and hydrolyzed with acid. The tritiated valine (0.2 μCi) thus produced was again exposed to L- and D-amino acid oxidases. The results clearly show that only the tritium atom at C-3 of the L-[2,3-^{3}H]valine is retained during its incorporation into ACV, for the tritium content of the valinyl residue (7000 cpm), derived from ACV, was about the same as that of the α-ketovaleric acid (6750 cpm), obtained upon oxidation with D-amino acid oxidase. Although the intimate details of the epimerization of the α-center of valine remain to be established, our data are incompatible with reaction mechanisms involving α,β-dehydrovalinyl intermediates or direct internal hydride shift, common among hydroxy acid racemases.[19]

These results, and the observation that tritiated ACV, labeled at the α-center of the D-valinyl moiety, was incorporated into penicillin N by protoplast lysates of *C. acremonium* with retention of tritium,[5] provide cogent evidence against the participation of free α,β-dehydrovalinyl tripeptide intermediate(s) in the formation of the penam nucleus.

Acknowledgment. We acknowledge the National Institutes of Health, AI-10519, for partial support of this research. The support of the Medical Research Council and the National Research Development Corporation is also gratefully acknowledged. We thank Professor Heinrich Schnoes for the mass spectrometric analyses of the β-lactam antibiotics.

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Received January 27, 1975

Chemiexcitation Mechanisms. The Role of Symmetry and Spin-Orbit Coupling in Diradicals

Sir:

For a Woodward-Hoffmann symmetry-forbidden pericyclic reaction, Figure 1a shows schematically the three potential energy surfaces connecting the ground (G), lowest