from the corresponding ketenimine are included for comparison.

Scheme I implies two additional methods of assessing the effect of the scavenging path on optical purity. Equation 2a gives the function needed to obtain the

$$\left(\frac{2(OP)}{1-(OP)}\right)_{DPPH} = \frac{k_s[DPPH]}{k_t + k_r} + \left(\frac{2(OP)}{1-(OP)}\right)_0 \quad (2a)$$

scavenging to rotation–tumbling rates. Figure 1 contains a plot of the observed points which yields the ratio as 0.0197 (mol/l.). Alternatively, eq 2 predicts that the function on the left should be independent of DPPH. These points are also included in Figure 1.

The present data show a very high degree of internal consistency in the ratios of rate constants estimated by independent comparisons with effective translational and rotational motions. Table II summarizes these results in terms of absolute rate constants by setting k_t as the reciprocal of the Debye–Stokes rotational correlation time for a particle with a 2 Å effective rotational radius.

The present results are in accord with the original[9] *cage* model for combination, as distinct from the more frequently noted geminate effect model,[10] in that no evidence for distinct primary and secondary pairs is observed. The scavenging results demonstrate the feasibility of trapping species with lifetimes of the order of 10^{-10} sec. The choice between decreased entropy of activation for the deaminatively formed hyponitrite (implicitly assumed in Table II) and a positive activation energy for the combination must await results of variable temperature studies of the optically active perester.

Acknowledgment. We are grateful to the National Science Foundation for financial support of this work.

(9) J. Frank and E. Rabinowitch, *Trans. Faraday Soc.*, **30**, 120 (1934); E. Rabinowitch and W. Wood, *ibid.*, **32**, 1381 (1936).
(10) Discussed in "Free Radicals," Vol. I, J. Kochi, Ed., Wiley, New York, N. Y., 1972.

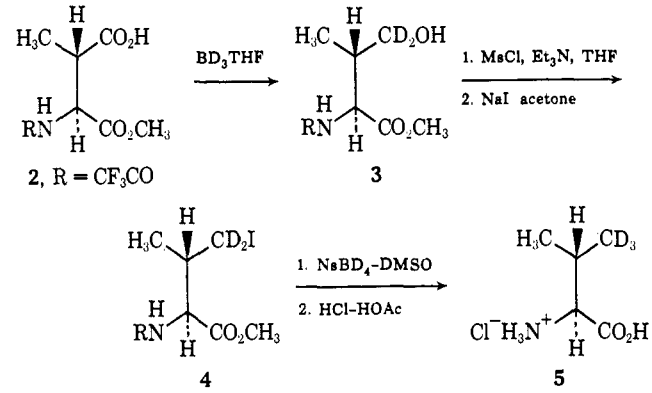
T. Koenig,* J. M. Owens
Department of Chemistry, University of Oregon
Eugene, Oregon 97403
Received January 18, 1974

Studies on the Incorporation of (2*S*,3*R*)-[4,4,4-$^2H_3$]Valine and (2*S*,3*S*)-[4,4,4-$^2H_3$]Valine into β-Lactam Antibiotics

Sir:

Previous studies have clearly shown the asymmetric incorporation of (2*S*,3*S*)-[4-^{13}C]valine[1] and (2*S*,3*R*)-[4-^{13}C]valine[2] into β-lactam antibiotics. As a sequel, we have undertaken the synthesis of chirally labeled CD_3-valines[3] to examine the stereochemical fate of the isopropyl hydrogenes. The α,β-dehydrovaline derivative of a tripeptide[4,5] has been proposed as a possible common intermediate in the biosynthesis of penicillin and cephalosporin. This paper reports studies on the fate of the diastereotopic deuterium-labeled methyls of L-valine in the course of their incorporation into penicillin N and cephalosporin C.

The synthetic sequence employed for the preparation of (2*S*,3*S*)-[4,4,4-$^2H_3$]valine (**1**) parallels that used for the synthesis of (2*S*,3*S*)-[4-^{13}C]valine[1] except that CD_3I[6] was substituted for $^{13}CH_3I$. The trifluoroacetamide methyl ester derivative of (2*S*,3*R*)-methylaspartic acid (**2**) was used as the starting material for the preparation of (2*S*,3*R*)-[4,4,4-$^2H_3$]valine (**5**). The deuterium was



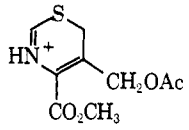
introduced *via* reduction of **2** with deuterated diborane yielding **3**, which was converted to the iodide, **4**, *via* mesylation followed by refluxing with sodium iodide in acetone. Reduction[7] of **4** with $NaBD_4$ in dimethyl sulfoxide followed by acidic hydrolysis afforded (2*S*,3*R*)-[4,4,4-$^2H_3$]valine (**5**) with a deuterium content of 78% d_3.

After incubation of **1** and **5** with washed cells of *Cephalosporium acremonium*, mutant C91,[8] for 10 hr, the resulting penicillin N and cephalosporin C were isolated,[1] converted into their respective *N*-acyl methyl ester derivatives,[9] and subjected to mass spectrometric analyses.[10] The results are summarized in Table I.

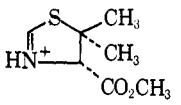
Consistent with our earlier incorporation experiments,[1] both penicillin N and cephalosporin C were enriched with deuterium to an extent of about 20–44%. Although several mass fragments[11] may be used in the calculation of isotopic ratios, the most intense of these are at *m/e* 174[12] for penicillin N and *m/e* 230 for cephalosporin C as shown in Table I. It is evident that the α-methyl of penicillin N derivative derived from **1** contained three deuteriums as clearly indicated by the very

(1) H. Kluender, C. H. Bradley, C. J. Sih, P. Fawcett, and E. P. Abraham, *J. Amer. Chem. Soc.*, **95**, 6149 (1973).
(2) N. Neuss, C. H. Nash, J. E. Baldwin, P. A. Lemke, and J. B. Grutzner, *J. Amer. Chem. Soc.*, **95**, 3797 (1973).
(3) For other syntheses of chirally labeled CD_3-valines see R. K. Hill, S. Yan, and S. M. Arfin, *J. Amer. Chem. Soc.*, **95**, 7857 (1973); D. J. Aberhart and L. J. Lin, *ibid.*, **95**, 7859 (1973).
(4) E. P. Abraham and G. G. F. Newton, *Biochem. J.*, **79**, 377 (1961).
(5) A. L. Demain, *Trans. N. Y. Acad. Sci.*, **25**, 731 (1963).
(6) CD_3I (>99%) was a product of Aldrich. The final product, **1**, had a deuterium content of >99%.
(7) Reduction of **4** using D_2 gas over 10% Pd/C at atmospheric pressure afforded valine with a deuterium content of only 51% d_3.
(8) B. Smith, S. C. Warren, G. G. F. Newton, and E. P. Abraham, *Biochem. J.*, **103**, 877 (1967).
(9) The crude penicillin N (79% pure by bioassay), isolated *via* the procedure previously described (ref 1), was treated with an excess of benzoyl chloride and potassium dibasic phosphate in acetone–water, followed by diazomethane. The *N*-benzylpenicillin N methyl ester was purified by tlc (pH 7.0 buffered silica gel) using ether–tetrahydrofuran (9:1) as the solvent system. The crude cephalosporin C was chromatographed on cellulose plates using 1-butanol–water–acetic acid (80:20:20); the purified cephalosporin C (90% pure by uv assay) was treated with acetic anhydride in phosphate buffer at pH 8–9. After the usual work-up, it was treated with diazomethane to yield *N*-acetylcephalosporin C methyl ester. In a control experiment, no isomerization of the Δ^3-cephem to the Δ^2-cephem derivative was observed under these conditions.
(10) Mass spectra were obtained with an AEI MS-9 mass spectrometer using direct probe introduction with an ion source temperature of 180–190°, electron potential of 70 eV and an ionizing current of 100 μA.
(11) Similar isotopic ratios of other fragments at *m/e* 485 and 366 for the cephalosporin C derivative and *m/e* 491 for the penicillin N were obtained.
(12) W. Richter and K. Biemann, *Monatsh. Chem.*, **95**, 766 (1964).

Table I. Isotopic Content of Cephalosporin C and Penicillin N Derivatives



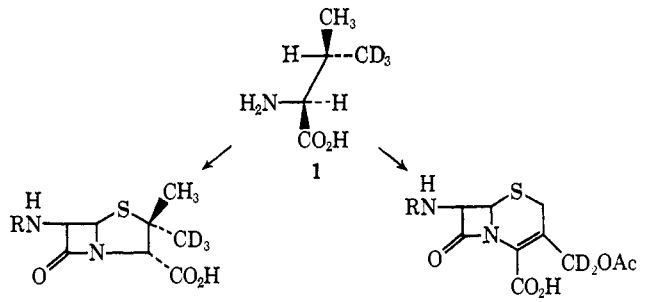
m/e 230



m/e 174

Valine precursor	Ceph C A d_0	d_1	d_2	d_3	d_4	d_5	d_6	Pen N B d_0	d_1	d_2	d_3	d_4	d_5	d_6
(2*S*,3*S*)-[4,4,4-$^2H_3$]-Valine[a]	63	4	30	3	0	0	0	56	0	1	43	0	0	0
(2*S*,3*R*)-[4,4,4-$^2H_3$]-Valine[b]	78	20	2	0	0	0	0	60	1	8	31	0	0	0
(2*RS*)[$^2H_6$]-Valine[c]	72	10	7	10	1	0	0	71	0	0	0	0	0	29

[a] The deuterium contents were 1% d_2 and 99% d_3. [b] 3% d_0, 20% d_2, and 77% d_3. [c] 95% d_6 and 5% d_5.

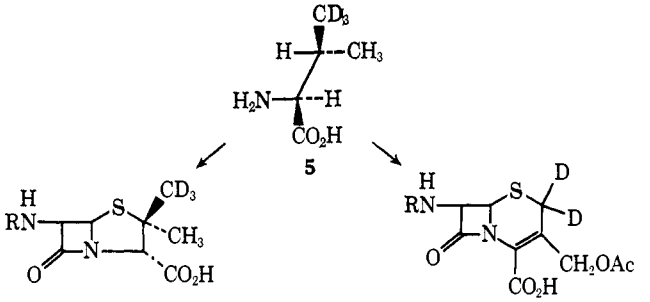


prominent (P + 3) peak at *m/e* 177. With cephalosporin C originating from **1**, an intense (P + 2) peak at *m/e* 232 was evident indicating that two deuterium atoms were retained at the exocyclic C-17 position.

A strong (P + 3) peak was observed with the penicillin N derivative from **5** again demonstrating that all three deuteriums in (2*S*,3*R*)-[4,4,4-$^2H_3$]valine were incorporated intact into the β-methyl group of the penam nucleus. However, mass spectrometric analyses of the cephalosporin C derivative derived from **5** did not give a clear picture as the results showed 77.7% d_0, 20.3% d_1, and 2.0% d_2 in the derivative suggesting that either one or two deuteriums may be incorporated into the endocyclic C-2 position of the cephem nucleus. In an attempt to acquire more accurate isotopic ratios, (3*RS*)-[$^2H_6$]valine (95% d_6) was prepared[13] with the objective of obtaining a cephalosporin C sample devoid of interferences from the natural abundances of (P + 1) and (P + 2) peaks, but, again, the results were inconclusive since low but significant quantities (1%) of (P + 4) and (9.8%) of (P + 3) peaks were noted.

Although one may envisage a biosynthetic pathway whereby the (*R*)-methyl group of L-valine is metabolized to the oxidation state of an aldehyde, which then undergoes subsequent ring closure, it is also possible that this ambiguity may be the result of intermolecular deuterium scrambling in the mass spectrometer. Thus, the cephalosporin C derived from (2*RS*)-[$^2H_6$]valine was converted into its *N*-benzoyl derivative shifting the C-14 proton resonance signal further downfield and clearly separated from the AB quartet representing the C-2 methylenes at δ 3.42 and 3.69. Careful quantitative pmr[14] analyses of this *N*-benzoylcephalosporin C revealed no differences in the areas of these C-2 protons, suggesting that the endocyclic methylenes at C-2 possessed two deuteriums.

To answer this question more definitively, an incubation with washed cells of *C. acremonium* was carried out in 80% D_2O.[15] The pmr spectrum of the resulting cephalosporin C showed the complete absence of deuterium at the C-2 position as evidenced by the complete symmetry of the AB quartet,[16] thereby establishing that both protons at C-2 originated from the (3*R*)-methyl of L-valine.



These experimental data make it unlikely that the formation of the Δ^3-cephem nucleus proceeds *via* a Δ^2-cephem intermediate. Further, they are in accord with, although they do not prove, the participation of an α,β-dehydrovalinyl derivative in the biosynthesis of the penam nucleus.

Acknowledgment. Grateful acknowledgment is made to the National Institutes of Health for their generous support of this research through Grant AI-10,519. The support of the Medical Research Council and the National Research Development Corporation is also gratefully acknowledged. We thank Professor Howard Whitlock for some of the nmr analyses and Dr. Roger Foltz of Battelle for many of the mass spectrometric analyses of the β-lactam antibiotics.

(13) N. F. Albertson, *J. Amer. Chem. Soc.*, **72**, 1396 (1950).

(14) Nuclear magnetic resonance spectra were obtained on a Bruker HX-90E spectrometer. Ten per cent D_2O served as the lock signal, and chemical shifts are given in parts per million relative to tetramethylsilane.

(15) B. C. Carlstedt, H. L. Crespi, M. I. Blake, and J. J. Katz, *J. Pharm. Sci.*, **60**, 1661 (1971), reported the failure to label the methyl groups of benzylpenicillin from D_2O during fermentation.

(16) The stereospecific incorporation of deuterium at this carbon during biosynthesis would result in an asymmetric quartet. Also, integrals of the C-2 protons at δ 3.88 and 4.12 revealed no significant differences. Substantial amounts of deuterium were incorporated into the C-7, C-6, the acetoxyl group, and the α-aminoadipic acid side chain of cephalosporin C.

Harold Kluender, Fu-Chih Huang, Alan Fritzberg
Heinrich Schnoes, Charles J. Sih*
School of Pharmacy and Department of Biochemistry
University of Wisconsin
Madison, Wisconsin 53706

Patricia Fawcett, E. P. Abraham
Sir William Dunn School of Pathology, University of Oxford
Oxford, England

Received April 8, 1974

Use of Copper Hexafluoroacetylacetonate for the Determination of the Absolute Configuration of Alcohols

Sir:

In the following, the methods for correlating the absolute configurations of 1,2- and 1,3-glycols with signs of the CD Cotton effects in the presence of Pr-