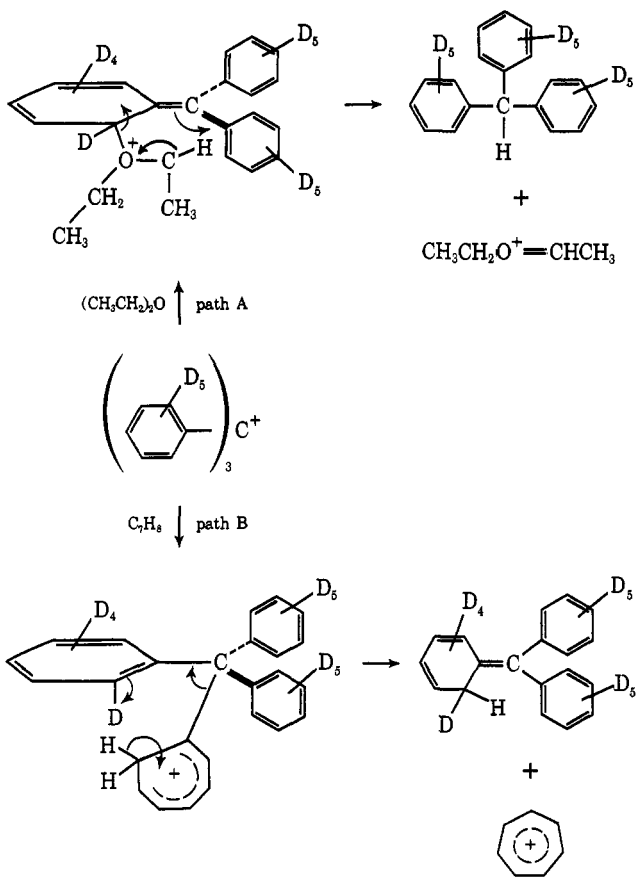

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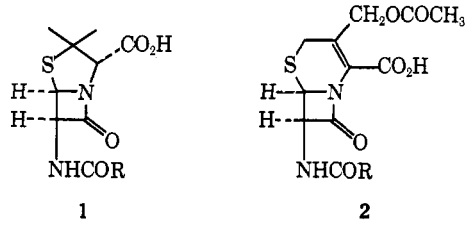
Use of Chiral Isopropyl Groups in Biosynthesis. Synthesis of (2*RS*,3*S*)-[4-^{13}C]Valine

Sir:

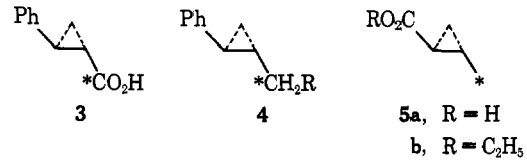
In spite of extensive efforts over the past two decades, the detailed biosynthetic pathway to the β-lactam antibiotics, penicillin and cephalosporin, is still unknown.[1] The evident relationship of the penam (**1**) and 3-cephem



1 **2**

(**2**) derivatives to the amino acids L-valine and L-cysteine has been established by appropriate incorporation experiments.[2] Since L-valine has been successfully incorporated into both systems, **1** and **2**,[2] we decided to use a valine with a chiral isotopic label (^{13}C) at the 4 position as a probe for the stereochemical fate of this isopropyl group, during conversion to the β-lactam products.

We have chosen the stereospecific, reductive opening of *trans*-2-methylcyclopropanecarboxylic ester (**5b**),[3] of known absolute configuration, as the method for generation of the chiral isopropyl group. Thus, carboxylation in THF of 2-phenylcyclopropylmagnesium bromide with [^{13}C]carbon dioxide[4] at −60° gave after esterification (diazomethane), equilibration to the trans isomer (potassium *tert*-butoxide in refluxing *tert*-butyl alcohol), and hydrolysis in methanolic potassium hydroxide at reflux, [^{13}C]carboxyl-labeled *trans*-2-phenylcyclopropanecarboxylic acid (85%), which after three recrystallizations as the quinine salt yielded (+)-*trans*-(1*S*,2*S*)-([1-^{13}C]carboxy)-2-phenylcyclopropane (**3**) of 100% optical purity, $[\alpha]^{22}D$ +314.0° (*c* 1.776, EtOH).[5] Reduction of the derived methyl ester (diazomethane)



3 **4** **5a**, R = H; **b**, R = C_2H_5

with lithium aluminum hydride in ether at 0° gave the alcohol **4** (R = OH, 95%), $[\alpha]^{22}D$ +89.3° (*c* 3.72, EtOH),[6] which upon mesylation at −25° in methylene chloride and reduction (lithium aluminum hydride, ether, −40°) gave (+)-*trans*-(1*S*,2*S*)-([1-^{13}C]methyl)-2-phenylcyclopropane (69%) (**4**, R = H), bp 76° (20 mm), $[\alpha]^{25}D$ +96° (*c* 3.72, EtOH). Destructive ozonization of this hydrocarbon in carbon tetrachloride at room temperature gave after distillation (47%) (+)-*trans*-(1*S*,2*S*)-([1-^{13}C]methyl)-2-cyclopropanecarboxylic acid (**5a**), bp 98° (18 mm), $[\alpha]^{19}D$ +99.2° (*c* 3.09, EtOH). Following esterification (diazoethane) and distillation (+)-*trans*-(1*S*,2*S*)-([1-^{13}C]methyl)-2-cyclopropanecarboxylic acid ethyl ester (**5b**), bp 80° (70 mm), was obtained (64%): ^{1}H nmr ($CDCl_3$)[7] δ 1.14 (dd, $J(^{13}C-^1H)$ = 128 and $J(^1H-^1H)$ = 5.5 Hz). Reductive cleavage of this substance with lithium in liquid ammonia (−78°) gave after preparative vpc pure (3*S*)-[4-^{13}C]-3-methylbutyric acid ethyl ester (**6**) in 18% yield;[8] ^{1}H nmr ($CDCl_3$) δ 0.96 (m, $J(^{13}C-^1H)$

(1) P. A. Lemke and D. R. Brannon in "Cephalosporins and Penicillins," E. H. Flynn, Ed., Academic Press, New York, N. Y., 1972, p 370.

(2) (a) H. R. V. Arnstein and M. E. Clubb, *Biochem. J.*, **65**, 618 (1957); (b) H. R. V. Arnstein and P. T. Grant, *ibid.*, **57**, 353, 360 (1954); (c) S. C. Warren, G. G. F. Newton, and E. P. Abraham, *ibid.*, **103**, 902 (1967); (d) E. P. Abraham, G. G. F. Newton, and S. C. Warren, *I. A. M. Symp. Appl. Microbiol., Tokyo*, **6**, 79 (1964).

(3) The literature contains a number of examples of the stereospecific, reductive cleavage of acylcyclopropanes: *e.g.*, H. O. House and C. J. Blankley, *J. Org. Chem.*, **33**, 48 (1968); W. G. Dauben and E. J. Deving, *ibid.*, **31**, 3794 (1966).

(4) Obtained from Monsanto Research Corp., Miamisburg, Ohio, 92% isotopic purity.

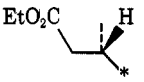
(5) The optically pure acid (**3**) is reported to have $[\alpha]^{22}D$ +311.7° (*c* 1.78, EtOH) by Y. Inouye, T. Sugita, and H. M. Walborsky, *Tetrahedron*, **20**, 1695 (1964). These authors have correlated this substance with *trans*-1,2-dimethylcyclopropane, of known configuration; *cf.* W. von E. Doering and W. Kirmse, *Tetrahedron*, **11**, 272 (1960).

(6) This sequence is essentially that described by T. Sugita and Y. Inouye, *Bull. Chem. Soc., Jap.*, **39**, 1075 (1966).

(7) All spectra are 100 MHz; unless mentioned otherwise, only resonances resulting from direct interaction of ^{13}C and ^{1}H are reported in this communication.

(8) Examination of the reaction mixture by vpc indicated a yield of 40–50% of the desired product. All substances have been examined by vpc and shown to be homogeneous.

= 125 and $J(^1H-^1H)$ = 6.4 Hz) and δ 0.96 ($^{12}CH_3$, m, $J(^{13}C-^1H)$ = 5.3 and $J(^1H-^1H)$ = 6.4 Hz). Integration indicated the presence of 92% ^{13}C and 8% ^{12}C in the methyl group. The free acid, obtained by saponification (92%, methanolic potassium hydroxide at reflux),



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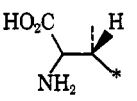

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^1H nmr ($CDCl_3$) δ 0.96 (dd, $J(^{13}C-^1H)$ = 125 and $J(^1H-^1H)$ = 6.4 Hz), was converted to the α-bromo derivative[9] (50–60%): ^1H nmr ($CDCl_3$) δ 1.10 (m, $J(^{13}C-^1H)$ = 10.5 Hz) and δ 4.1 (m, $J(^{13}C-^1H)$ = 3.0 and $J(^1H-^1H)$ = 7.7 Hz). Aminolysis[10] of the crude compound afforded (2*RS*,3*S*)-[4-^{13}C]valine (**7**) purified by ion-exchange chromatography (Bio-Rad AG 50W-x8, H^+ 50–100 mesh) (56%). ^1H nmr[11] (D_2O) δ 1.42 m ($J(^{13}C-^1H)$ = 126 and $J(^1H-^1H)$ = 6.9 Hz), δ 2.72 m ($J(^{13}C-^1H) \sim 10$ Hz), and δ 4.04 m ($J(^{13}C-^1H)$ = 4.2 and $J(^1H-^1H)$ = 4.2 Hz). The amino acid, crystallized from ethanol, was shown to be identical with authentic D,L-valine by comparison of tlc, X-ray powder data, vpc, and mass spectra with expected increases of *m/e* due to ^{13}C. The estimated optical and isotopic purity is 100 and 92% respectively. This material was used in the labeling of cephalosporin C and penicillin V.[12]

Acknowledgments. We wish to thank the following members of the Lilly Research Laboratories for their invaluable help: Dr. H. E. Boaz (interpretation of the nmr spectra), Mr. T. K. Elzey (recording of the nmr spectra), Mr. R. D. Miller (optical rotation), Mr. H. L. Hunter (vpc data and separation of chiral ethyl isovalerate), Messrs. D. C. Hunden and F. E. Heiliger (preparation of phenylcyclopropyl bromide) and J. B. Campbell (aminolysis).

(9) This was carried out with bromine and phosphorus trichloride according to C. S. Marvel, "Organic Syntheses," Collective Volume III, Wiley, New York, N. Y., 1955, p 848.

(10) Conducted in a Parr bottle with liquid ammonia at room temperature; *cf.* N. D. Cheronis and K. H. Spitzmueller, *J. Org. Chem.*, **6**, 349 (1941), who carried out the reaction in a sealed tube.

(11) The ^{12}C and ^{13}C isopropyl multiplets show an isomeric difference of 0.04 ppm arising from the D,L center in the α-bromo acid and 0.05 ppm in (2*RS*,3*S*)-[4-^{13}C]valine.

(12) N. Neuss, C. H. Nash, J. E. Baldwin, P. A. Lemke, and J. B. Grutzner, *J. Amer. Chem. Soc.*, **95**, 3797 (1973).

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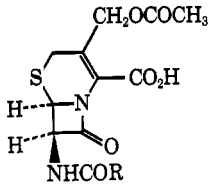
Incorporation of (2*RS*,3*S*)-[4-^{13}C]Valine into Cephalosporin C

Sir:

Previous analyses of the cmr spectra of cephalosporin C, biosynthesized from sodium [1-^{13}C]- and [2-^{13}C]-acetate,[1] and D,L-[1-^{13}C]- and [2-^{13}C]valine,[2] suggested that valine with a chiral ^{13}C label at C-4 would provide a simple and unequivocal determination of the fate of the isopropyl group during the formation of β-lactam antibiotics. The result of such an experiment could shed light on the proposal that 3-cepham (**1**) antibiotics



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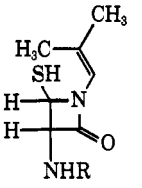

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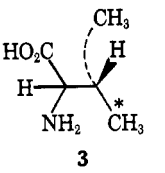

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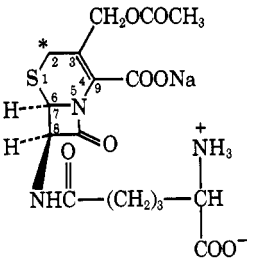

4

could be derived[3,4] from a common α,β-dehydro valine derivative of tripeptide (**2**).

Submerged cultures of *Cephalosporium acremonium*, a superior antibiotic producing mutant, M 8650-3,[5] were grown at 25° on a rotary shaker (250 rev/min) in a complex medium.[6] Cephalosporin C was labeled with (2*RS*,3*S*)-[4-^{13}C]valine (**3**)[7] after 46, 54, 78, and 90 hr of incubation as described before.[1,2] Fermentation broth was collected by filtration after 115 hr. Cephalosporin C was purified and crystallized as the sodium salt[8] (**4**).

Comparison of the proton noise decoupled, cmr spectrum of this material with that of unlabeled cephalosporin C[1] showed *a fivefold enhanced intensity at the C-2 resonance (8.6% of incorporation if one assumes that only* L*-valine is being utilized)*[9] *without any other detectable changes in spectrum.* It should be emphasized that even a 5% increase in intensity of resonance would be noticeable under conditions used for the recording of these spectra.

The result of this feeding experiment clearly demonstrates the value of a ^{13}C chiral label in valine as a precursor in biosynthesis of cephalosporin C and the unique advantage of cmr spectroscopy over the conventional tracer technique.

Biogenetic consequences of this unequivocal outcome of our experiment are of great significance in the

(1) N. Neuss, C. H. Nash, P. A. Lemke, and J. B. Grutzner, *J. Amer. Chem. Soc.*, **92**, 1338 (1970).

(2) N. Neuss, C. H. Nash, P. A. Lemke, and J. B. Grutzner, *Proc. Roy. Soc., Ser. B*, **179**, 335 (1971).

(3) E. P. Abraham and G. G. F. Newton, *Biochem. J.*, **79**, 377 (1961).

(4) A. L. Demain, *Trans. N. Y. Acad. Sci.*, **25**, 731 (1963).

(5) D. W. Dennen and D. D. Carver, *Can. J. Microbiol.*, **15**, 175 (1969).

(6) P. A. Lemke and C. H. Nash, *Can. J. Microbiol.*, **17**, 255 (1971).

(7) J. E. Baldwin, J. Löliger, W. Rastetter, N. Neuss, L. L. Huckstep, and N. De La Higuera, *J. Amer. Chem. Soc.*, **95**, 3796 (1973).

(8) P. W. Trown, E. P. Abraham, G. G. F. Newton, C. W. Hale, and G. A. Miller, *Biochem. J.*, **84**, 157 (1962).

(9) An unequivocal decision in regard to the rate of incorporation of valine cannot be reached due to a number of complicating factors which have been considered in the biosynthesis of *Cephalosporium* (ref 8 and references cited therein).