literature[20-25] and provides reasonable analogy for the above suggestion.

It is interesting at this point to compare the photochemistry of **1** with that of the previously reported parent lactone, benzofuran-2(3*H*)-one (**11**). As noted by Chapman,[3] **11** give *o*-hydroxybenzyl methyl ether (**12**) upon irradiation in methanol. We have determined that the quantum yield for formation of **12** is 0.20 with 2537-Å light. Under optimal conditions **1** is converted to the analogous product (*i.e.* **2**) with a quantum efficiency of only 0.058. The difference in quantum yields in these two systems is apparently due to a number of competing photoprocesses which deactivate the excited state(s) of **1**. In addition to the visible modes of decay which produce **2** and **3**, an "invisible" process resulting in enolization of **1** decreases the efficiency of product formation. We could find no evidence for a similar process in the photochemistry of **11** and were unable to detect any significant amount of salicylaldehyde when the irradiation of **11** was carried out in the presence of oxygen. It would appear as though the phenyl substituent not only controls the tautomeric composition of the lactone but also markedly enhances the photoenolization route. The wavelength and solvent effects which we have noted for **1** suggest that tautomeric forms of carbonyl derivatives may yield diverse and interesting photochemistry. We are continuing to examine these effects and will report our complete findings at a later date.

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(20) J. Lemaire, *J. Phys. Chem.*, **71**, 2653 (1967).
(21) R. Bishop and N. K. Hamer, *J. Chem. Soc. C*, 1197 (1970).
(22) Y. Kanda, I. Stainislaus, and E. C. Lim, *J. Amer. Chem. Soc.*, **91**, 5085 (1969).
(23) R. G. Zepp and P. J. Wagner, *ibid.*, **92**, 7466 (1970).
(24) N. J. Turro and T. J. Lee, *ibid.*, **92**, 7467 (1970).
(25) N. C. Yang and S. L. Murov, *J. Chem. Phys.*, **45**, 4358 (1966).
(26) Alfred P. Sloan Foundation Fellow, 1968–1972; National Institutes of Health Special Postdoctoral Fellow, 1972–1973
(27) National Institutes of Health Postdoctoral Fellow, 1971–1972.

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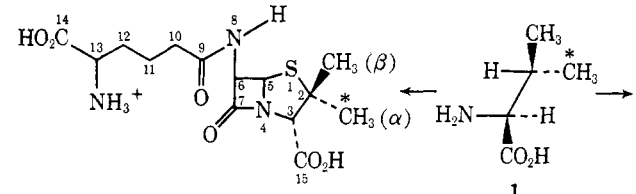
Synthesis and Incorporation of (2*S*,3*S*)-[4-^{13}C]Valine into β-Lactam Antibiotics

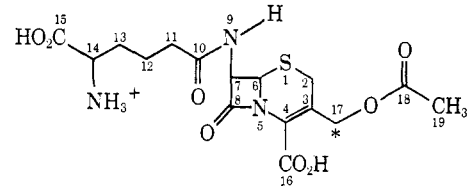
Sir:

The entire carbon skeleton of L-valine has been shown to be incorporated into the thiazolidine ring of penicillin and the dihydrothiazine ring of cephalosporin.[1] We herein report the synthesis of (2*S*,3*S*)-[4-^{13}C]valine (**1**) and results which shed light on its asymmetric incorporation into penicillin N (**2**) and cephalosporin C (**3**) by *Cephalosporium acremonium*, mutant C91.[2]

(1) P. A. Lemke and D. R. Brannon in "Cephalosporins and Penicillins," E. H. Flynn, Ed., Academic Press, New York, N. Y., 1972, pp 370–437.
(2) B. Smith, S. C. Warren, G. G. F. Newton, and E. P. Abraham, *Biochem. J.*, **103**, 877 (1967).



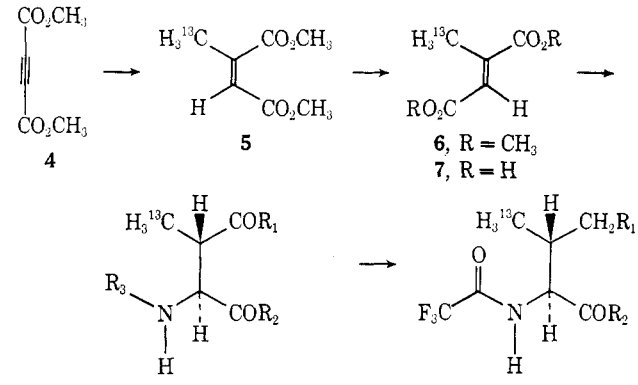
2 **1**



3

Scheme I outlines the reaction sequences used for

Scheme I



4 **5** **6**, R = CH$_3$; **7**, R = H

8, R$_3$ = H; R$_1$ = R$_2$ = OH
9, R$_3$ = CF$_3$CO; R$_1$ = R$_2$ = >O
10, R$_3$ = CF$_3$CO; R$_1$ = OH; R$_2$ = OCH$_3$
11, R$_3$ = CF$_3$CO; R$_1$ = OCH$_3$; R$_2$ = OH

12, R$_1$ = OH; R$_2$ = OCH$_3$
13, R$_1$ = OMs; R$_2$ = OCH$_3$
14, R$_1$ = I; R$_2$ = OCH$_3$
15, R$_1$ = H; R$_2$ = OCH$_3$

the synthesis of (2*S*,3*S*)-[4-^{13}C]valine. Reaction of **4** with [^{13}C]methyl copper[3] at −78° afforded dimethyl [*methyl*-^{13}C]citraconate (**5**) (70% yield), which was isomerized[4] by light and a trace of bromine to a mixture of **6** and **5** (95:5). Acid hydrolysis of **6** yielded **7** (81% from **5**). Exposure of [*methyl*-^{13}C]mesaconic acid (**7**) to β-methylaspartase[5] gave (2*S*,3*R*)-3-[^{13}C]methylaspartic acid (**8**) in 88% yield after recycling of recovered **7**. After quantitative conversion of **8** into its cyclic anhydride trifluoroacetamide derivative,[6] **9** was stirred in anhydrous methanol to give an isomeric mixture of **10** and **11** (8:2).[7] Treatment of **10** with diborane in THF at 0° afforded **12**, which was immediately converted to **13**. The mesylate **13** was refluxed with sodium iodide in acetone to afford **14** (66% from **8**). Hydrogenolysis of **14** in methanol–triethylamine (2:1) over 10% Pd/C at atomospheric pressure gave **15** in 92% yield. Acid hydrolysis of **15** gave (2*S*,3*S*)-[4-^{13}C]valine hydrochloride[8] in 84% yield: [α]^{25}D +25.2°

(3) [^{13}C]Methyl iodide (90% isotopic purity, Merck & Co., Inc.) was allowed to react with lithium wire in ether at 0° under argon. The resulting methyllithium solution was diluted with THF and cooled to −78° before 1.1 equiv of a solution of cuprous iodide (29% w/w) in diisopropyl sulfide was added dropwise.
(4) V. C. F. Langworthy, *Ann.*, **304**, 145 (1899).
(5) H. A. Barker and R. D. Smyth, *Biochem. Prep.*, **8**, 89 (1962).
(6) F. Weygand and M. Reiher, *Chem. Ber.*, **88**, 26 (1955).
(7) F. Weygand and R. Geiger, *Chem. Ber.*, **90**, 634 (1957).
(8) The chromatographic behavior, proton magnetic resonance (pmr), and infrared spectra were all consistent with the assigned structure. Satisfactory carbon–hydrogen analyses were obtained for all the compounds reported herein.

(*c* 2, 10% HCl); cmr[9] (H_2O–D_2O, 9:1, neutralized with NaOH) δ 17.6 ppm (methyl) was strongly labeled whereas δ 18.8 ppm (methyl) was unlabeled.

The stereospecificity of our synthesis is supported by the cmr spectrum of the final product, which exhibited only one ^{13}C-labeled methyl signal. The assignment of the 2*S*,3*S* configuration is consistent with the mode of synthesis from (2*S*,3*R*)-3-[^{13}C]methylaspartic acid.[10] The configuration of the β center was correlated with asymmetrically labeled [4-$^2H_3$]valine prepared by Professor R. K. Hill,[11] who observed that the (*R*)-methyl possessed a lower chemical shift than the (*S*)-methyl of valine. When unlabeled **14** was reduced with deuterium gas, the pmr signal of the lower field (*R*)-methyl of the resulting valine was reduced in intensity indicating that the γ-carboxyl group in enzymatic 3-methylaspartic acid corresponds to the (*R*)-methyl of L-valine, thus confirming the 3*S* assignment of the ^{13}C-labeled valine.

(2*S*,3*S*)-[4-^{13}C]Valine (from 100 mg of hydrochloride neutralized with NaOH) was added in eight portions to washed cells of *C. acremonium* (60 g) in shake flasks during 10 hr. The filtrate was freeze-dried and the residue was chromatographed over a Sephadex G-25 (fine grade) column. Elution of the column with isopropyl alcohol–H_2O (7:3) afforded 25 mg of crude penicillin N (79% pure by bioassay). In a similar experiment, 4.1 mg of crude cephalosporin C was obtained.

The cmr spectrum of the cephalosporin C sample showed that the methylene carbon at C-17 (δ 65.0 ppm)[12] was very strongly labeled (signal:noise, 30:1). None of the contaminant signals[13] corresponded to carbons of cephalosporin C. The cmr spectral data of penicillin N are listed in Table I. The chemical-shift assignments of the side-chain carbons of penicillin N were made by a direct correlation with those of cephalosporin C.[12] Other signals were assigned by correlations with published penicillin cmr spectra.[14] The α-methyl carbon appeared to be labeled >20%. No significant amount of label above natural abundance was observed of the β-methyl carbon. The pmr spectrum of the ^{13}C-labeled penicillin N further corroborates this conclusion, for the upfield methyl signal, δ 1.32 ppm (α-methyl by comparison with published data[15]), is reduced in intensity, and the two ^{13}C-satellite signals ($J(^{13}C–^1H)$ = 128.4 Hz) are intense.

These experimental data clearly show that the (2*S*,3*S*)-[4-^{13}C]valine is incorporated into penicillin N with retention of configuration. In the case of cephalosporin C, the ^{13}C label is located in the exocyclic methylene carbon (C-17).[16]

Table I. ^{13}C Chemical-Shift Assignments[a] of Some β-Lactam Antibiotics

Assignment	Chemical shifts: Penicillin N	Methyl-6-acetamido penicillinate[c]	Cephalosporin C: Present work	Lit.[d]
C-11	21.8		35.5	34.7
C-α	27.3	26.1		
C-β	30.9[b]	30.0		
C-12	30.7[b]	30.0	21.8	20.2
C-10	35.4			
C-13	55.4		30.7	29.7
C-6	58.8	58.3		
C-2	65.1	63.6		
C-5	67.4	67.1		
C-3	74.0	69.7		
C-7	175.3[b]			
C-9	175.4[b]			
C-14	175.2[b]		55.5	54.9
C-15	176.7[b]			

[a] Chemical shifts were measured relative to internal *p*-dioxane and corrected to $(CH_3)_4Si$ as internal reference by the relationship $\delta_c(CH_3)_4Si = \delta(p\text{-dioxane}) + 67.4$ (J. B. Stothers, "Carbon-13 NMR Spectroscopy," Academic Press, New York, N. Y., 1972, p 49). [b] The relative assignments have not been made of these carbons. [c] Reference 14. [d] Reference 12.

Acknowledgment. This investigation was supported by research grants from the National Institutes of Health (AI-10,519). The authors are indebted to Professor Richard K. Hill, who kindly informed us in detail of his synthesis and stereochemical assignments of asymmetrically labeled valine-*4-d*$_3$ in advance of publication, Professor D. Arigoni for valuable suggestions, and Mr. John Usher for a sample of purified unlabeled penicillin N. We acknowledge the support of the Graduate School of the University of Wisconsin for purchase of the nmr spectrometer and pulse Fourier transform accessories.

(9) The carbon magnetic resonance (cmr) spectra were recorded at 22.63 MHz in the Fourier transform mode of operation with proton broad band decoupling on a modified Bruker HX-90E using 10-mm sample tubes. Ten per cent D_2O served as the lock signal and all chemical shifts are given in parts per million relative to tetramethylsilane using *p*-dioxane as the internal standard. The natural abundance cmr spectra of valine were reported by W. Horsley, H. Sternlicht, and J. S. Cohen, *J. Amer. Chem. Soc.*, **92**, 680 (1970).

(10) H. A. Barker, R. D. Smyth, E. J. Wawszkiewicz, M. N. Lee, and R. M. Wilson, *Arch. Biochem. Biophys.*, **78**, 468 (1958).

(11) Private communication.

(12) A natural abundance cmr spectrum of cephalosporin C was published by N. Neuss, C. H. Nash, P. A. Lemke, and J. B. Grutzer, *J. Amer. Chem. Soc.*, **93**, 2337, 5314 (correction) (1971).

(13) The signals of the contaminants were at δ 27.3 (α-CH_3 of penicillin N), 28.6 (penicillin N decomposition product), and 73.3 ppm (unknown).

(14) R. A. Archer, R. D. G. Cooper, and P. V. Demarco, *Chem. Commun.*, 1291 (1970).

(15) See ref 1, pp 686–703.

(16) Since the submission of this manuscript, two communications on this same topic have appeared (see J. E. Baldwin, J. Löliger, W. Rastetter, N. Neuss, L. L. Huckstep, and N. De La Higuera, *J. Amer. Chem. Soc.*, **95**, 3796 (1973); N. Neuss, C. H. Nash, J. E. Baldwin, P. A. Lemke, and J. B. Grutzner, *ibid.*, **95**, 3797 (1973)). Dr. Neuss has kindly informed us that the correct nomenclature of their synthetic chiral valine is (2*RS*,3*R*)-[4-^{13}C]valine. It is gratifying to note the complementary results of the two incorporations (2*S*,3*R*)-[4-^{13}C]valine (Baldwin) and (2*S*,3*S*)-[4-^{13}C]valine (this paper).

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Stereochemical Dependence of the Chemical-Shift Isotope Effect

Sir:

Dependence of the chemical shift of a nucleus on the isotopic identity of neighboring nuclei was initially observed in 1953 for the case of the hydrogen molecule.[1] Despite an increasing amount of empirical data, the cause of isotope shifts in polyatomic mole-

(1) T. F. Wimett, *Phys. Rev.*, **91**, 476 (1953).