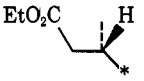
= 125 and $J(^1H-^1H)$ = 6.4 Hz) and δ 0.96 ($^{12}CH_3$, m, $J(^{13}C-^1H)$ = 5.3 and $J(^1H-^1H)$ = 6.4 Hz). Integration indicated the presence of 92% ^{13}C and 8% ^{12}C in the methyl group. The free acid, obtained by saponification (92%, methanolic potassium hydroxide at reflux),



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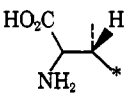

7

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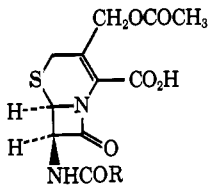
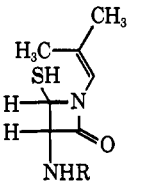
Incorporation of (2*RS*,3*S*)-[4-^{13}C]Valine into Cephalosporin C

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



1



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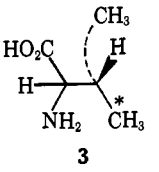

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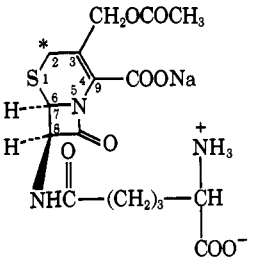


4

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).

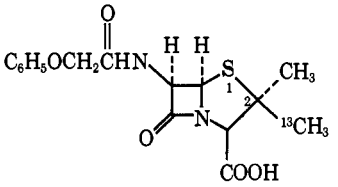
(9) An unequivocal decision in regard to the rate of incorporation of valine cannot be reached due to a number of complicating factors which have been considered in the biosynthesis of *Cephalosporium* (ref 8 and references cited therein).

understanding of the formation of this unique class of secondary metabolites; *e.g.*, derivation of cephalosporin C from a common α,β-dehydro valine derivative of tripeptide[3,4] and/or the biological importance of a "three-point combination" between the symmetrical substrate and the enzyme.[10,11]

Acknowledgments. We gratefully acknowledge the invaluable help of Mr. R. L. Pieper (fermentation and labeling experiment) and Mr. L. L. Huckstep and Mrs. N. De La Higuera (isolation of crystalline cephalosporin C sodium salt).

(10) A. G. Ogston, *Nature (London)*, **162**, 963 (1948). In this case for instance, one could postulate the existence of a stereochemically fixed complex involving an enzyme and only one of the methyl groups with the other available for transformation necessary for the ring closure.

(11) Since submission of this paper we have also completed labeling experiment of penicillin V (**5**) in a submerged culture of *P. chrysogenum*



5

(P. A. Lemke, C. H. Nash, and S. W. Pieper, *J. Gen. Microbiol.*, in press) with (2*RS*,3*S*)-[4-^{13}C]valine. Penicillin V was purified and crystallized as the potassium salt. Comparison of the cmr spectrum of this material with that of unlabeled penicillin V (R. A. Archer, R. D. G. Cooper, P. V. Demarco, and L. R. F. Johnson, *Chem. Commun.*, 1291 (1970)) *showed a (1.7 ± 0.2)-fold enhanced intensity of the β-CH$_3$ resonance at C-2 without any other detectable changes in the spectrum.* This corresponds to 1.6% of incorporation if one assumes that only L-valine is being utilized.

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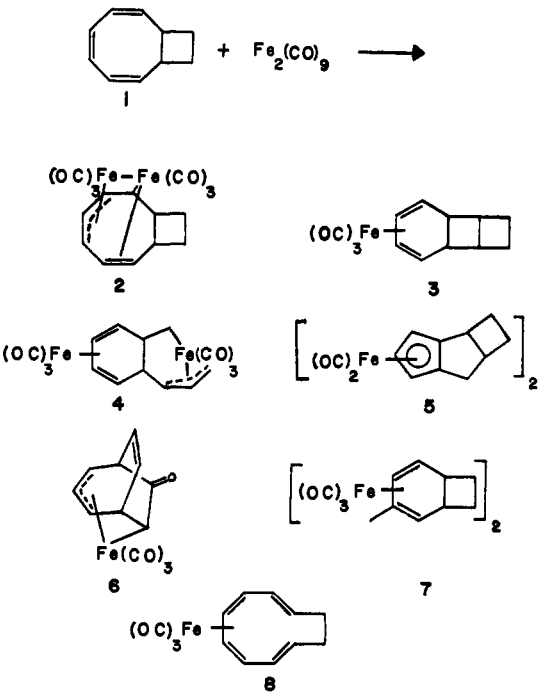
Further Exotic Products from the Reaction of Diiron Nonacarbonyl with Bicyclo[6.2.0]deca-2,4,6-triene. X-Ray Crystallography as a Practical Means of Cheap, Rapid, and Definitive Analysis

Sir:

We wish to report here a study which merits this mode of communication for two reasons. First because at least some of the new compounds described are intrinsically of considerable interest. Second, because in separate, detailed, conventional reports on the different compounds, which will appear in due course, probably in specialist journals, it would not be possible to present to a broad spectrum of readership an overview of the tactical approach employed which, we think, is both novel and uniquely powerful. While no single logical step or technique used is in itself new, the systematic and consistent way in which we proceeded is demonstrably powerful and is, to our knowledge, unprecedented.

The isolation of four products from the reaction of bicyclo[6.2.0]deca-2,4,6-triene (**1**) with $Fe_2(CO)_9$ has been reported.[1] The identities of two (**2** and **3**) of them were inferred from analytical and spectroscopic data[1] and these structures have since been confirmed crystallographically.[2-4] The structure of **4** was not deduced[1] from the analytical and spectroscopic data; it has since been found by crystallography to be as shown. The yields of **5** are minute but highly



reproducible; the quantity from any single reaction is too small for analysis for adequate spectroscopic characterization.[5] Thus, recourse to X-ray crystallographic characterization[6] was mandatory. Using a sample with an approximate mass of 50 μg the structure was solved in an elapsed time of *ca.* 60 hr at a computing cost of $157 (see Table I).

In the meantime, we had reinvestigated the reaction under varied conditions, and it was found that still

(1) F. A. Cotton and G. Deganello, *J. Organometal. Chem.*, **38**, 147 (1972).

(2) F. A. Cotton, B. A. Frenz, G. Deganello, and A. Shaver, *J. Organometal. Chem.*, **50**, 227 (1973) (compound **2**).

(3) F. A. Cotton, B. A. Frenz, and J. M. Troup, manuscripts in preparation (compounds **3** and **4**).

(4) Actually, the correct structure of **2** was *not* obtained from the spectroscopic data prior to the X-ray work, because the molecule is fluxional and has a surprisingly low coalescence temperature. However, further low-temperature nmr work subsequent to the X-ray work did provide spectroscopic evidence sufficient to yield the correct structure. Of course, the spectroscopically inferred structure of **3** was incomplete in that the stereochemistry of ring fusion was not elucidated.

(5) It is unlikely that even with a full set of the conventional spectroscopic data the actual structure, including ring fusion geometry, of **5** could have been deduced anyway.

(6) In this and all subsequent structures, the crystals were examined and data collected on a Syntex P$\bar{1}$ computer-controlled diffractometer equipped with a graphite crystal monochromater. The structures were solved using a three-dimensional Patterson function to locate the metal atom(s). A subsequent least-squares cycle and difference Fourier synthesis generally revealed all of the remaining nonhydrogen atoms. The refinement to convergence was always achieved after three full-matrix least-squares cycles on all positional and isotropic thermal parameters.