matically, the former leading to olefin production and the latter to bond relocation.[22] Studies in progress are intended to provide information underlying the causative factors behind this contrasting behavior.

Acknowledgment. Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for their partial support of this research. We also thank Professor H. Shechter for a most stimulating discussion.

(22) A review of Ag^+ catalysis will soon appear: L. A. Paquette, *Accounts Chem. Res.*, in press.

(23) (a) National Science Foundation Graduate Trainee, 1970–1971; (b) National Institutes of Health Postdoctoral Fellow, 1970–1971.

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The Use of Carbon-13 Nuclear Magnetic Resonance (Cmr) Spectroscopy in Biosynthetic Studies. Incorporation of Carboxyl and Methyl Carbon-13 Labeled Acetates into Cephalosporin C

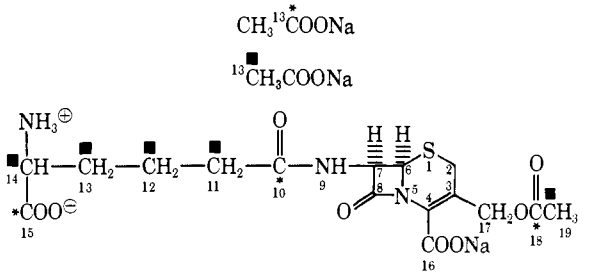
Sir:

The study of biosynthetic pathways leading to the formation of penicillins and cephalosporins has resulted in the understanding of the role of certain precursors involved in the formation of this class of microbial products.[1] Most of these studies have been conducted with the aid of radiotracer techniques. The availability of cmr spectroscopy prompted us to use this method in the study of incorporation of ^{13}C-labeled acetate into cephalosporin C. Cmr spectroscopy has been shown to be useful in the elucidation of biosynthetic pathways of microbial metabolites of a completely different type.[2,3]

Submerged cultures of *Cephalosporium acremonium*, a superior antibiotic producing mutant, M 8650-3,[4] were grown at 25° on a rotary shaker (250 rpm) in a complex medium.[5] In the first experiment, cephalosporin C was pulse labeled with [*1*-^{13}C]sodium acetate, and in the second with [*2*-^{13}C]sodium acetate[6] by the addition of aliquots of 1 ml of the aqueous solution[7] of the respective acetates to 70 ml of the broth in individual flasks after 46, 54, 66, 78, and 90 hr of incubation. Fermentation broth was collected by filtration after 115 hr. Cephalosporin C was purified and crystallized as the sodium salt.[8]

Chemical shifts of the 16 carbon atoms (Table I) in the natural abundance cmr spectrum of cephalosporin C–Na salt (I) were obtained from 25.2-MHz spectra using dioxane as internal reference. The assignment of the resonances was based on off-resonance and single-frequency proton decoupling experiments.[9] The carbon signals were divided into groups according to the number of directly bonded protons on the basis of the off-resonance spectra. Individual proton decoupling frequencies, obtained from the reported proton spectra of cephalosporin derivatives,[10] were then used to provide an unambiguous assignment of each of the carbons bearing protons. Thus, the assignment of C-2, C-6, C-7, C-11, and C-14 was uniquely defined by single frequency proton decoupling. Although C-12 and C-13 could not be differentiated from each other by this technique, their resonances were readily identified on the basis of chemical shifts and using appropriate models.[11]

Table I. Incorporation of ^{13}C-Labeled Acetates and Carbon-13 Chemical Shifts of Cephalosporin C (I)



Assignment[a]	Chemical shifts[b]	Rel intensities[c] Normal abundance	$^{13}CH_3$-COO$^-$Na$^+$ labeled	CH$_3$^{13}COO$^-$-Na$^+$ labeled
C-2	167.8	1.0	1.0	1.0
C-3	58.5	1.0	1.2	1.0
C-4	74.5	1.2	1.2	1.2
C-6	135.2	1.0	0.8	0.8
C-7	133.2	1.0	1.0	1.0
C-8	24.3	1.0	1.0	1.4
C-10	12.0	1.0	1.2	2.2
C-11	158.1	1.0	2.0	1.0
C-12	172.6	1.0	1.8	1.2
C-13	163.1	1.0	1.8	1.2
C-14	137.9	1.0	4.6	1.0
C-15	14.1	1.0	1.0	5.0
C-16	20.6	0.8	0.8	1.0
C-17	127.8	1.0	1.0	0.8
C-18	14.6	1.0	1.0	3.6
C-19	173.2	0.8	3.6	0.8

[a] This numbering system is different from the accepted numbering system for this class of compounds and is used uniquely for an easy identification of chemical shifts. [b] Spectra were recorded at 25.2 MHz on the Varian XL-100-15 using 12-mm tubes and the signal from a concentric tube containing acetone-d_6 as lock. All chemical shifts and intensities were obtained from *ca.* 1 *M* aqueous solutions containing ~10% dioxane as internal reference. Shifts are given in parts per million relative to CS_2 and have uncertainties of about ±0.1 ppm. [c] These are measured relative intensities from the spectra run under essentially identical instrumental conditions and accurate to ±0.2. The signal from acetone-d_6 provided a convenient check on relative intensities between samples. In order to obtain per cent incorporation, the figures in columns 4 and 5 should be corrected according to the formula % incorp = (100/62)·(I − 1), where I is the observed intensity.

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(5) Experimental details pertaining to condition of fermentation will be published elsewhere.

(6) [*1*-^{13}C]- and [*2*-^{13}C]sodium acetate, Merck Sharp & Dohme, Canada, 62–63 atom % ^{13}C.

(7) The solution was prepared by dissolving 20 mg of acetate in 1 ml of water and sterilizing by membrane filtration.

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(9) (a) M. Tanabe, T. Hamasaki, D. Thomas, and L. F. Johnson, *J. Amer. Chem. Soc.*, 93, 273 (1971); (b) J. D. Roberts, F. J. Weigert, J. I. Kroschwitz, and H. J. Reich, *ibid.*, **92**, 1338 (1970).

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Frequencies of C-3, C-4, C-17, and C-19 were located from their multiplicities, chemical shifts, and model systems.[12,13]

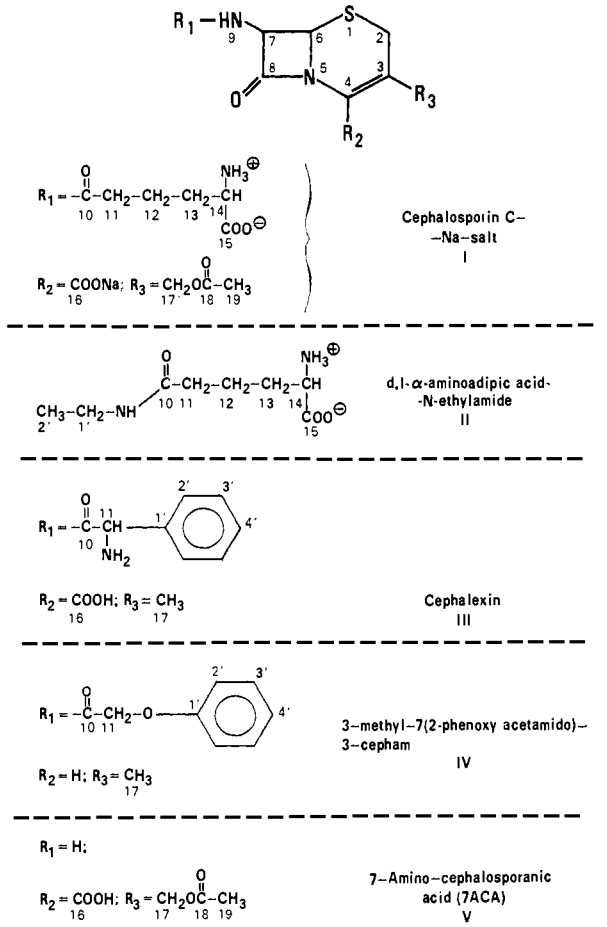
In order to facilitate additional assignments and to further substantiate the analysis given above, the cmr spectra of *dl*-α-aminoadipic acid ethylamide (II), cephalexin (III),[14] 3-methyl-7-(2-phenoxyacetamide)-3-cephem (IV),[15] and 7-aminocephalosporanic acid (V) were obtained (Table II). Single frequency and off-resonance proton decoupling were employed where necessary.

Table II. Carbon-13 Chemical Shifts of Cephalosporin Models and pH Dependence of Resonances in Cephalosporin C (I)

Assignment[a]	II[b]	III[b]	IV[c]	V[d]	*I*, pH 6.0	*I*, pH 3.0
C-2		164.5	164.7	167.0	167.8	167.3
C-3		49.9	75.8	60.4	58.5	60.1
C-4		68.4	69.8*	75.8	74.5	69.5
C-6		135.4	136.1	130.0*	135.2	134.9
C-7		133.9	134.3	133.7*	133.2	133.0
C-8		25.1	28.9*	29.0*	24.3	23.8
C-10	11.0*	12.9	22.9*		12.0	12.2
C-11	163.0	133.9	125.4		158.1	158.0
C-12	175.1				172.6	172.3
C-13	167.8				163.1	163.0
C-14	135.8				137.9	138.4
C-15	14.6*				14.1	15.1
C-16		19.5		24.0*	20.6	21.6
C-17		174.8	171.9	128.0	127.8	128.0
C-18				17.6	14.6	14.8
C-19				172.0	173.2	172.8
C-1′	157.7	63.6	34.5			
C-2′	181.9	61.3*	77.4			
C-3′		63.6*	62.3			
C-4′		62.1	73.9*			

[a] This numbering system is different from the accepted numbering system for this class of compounds and is used uniquely for an easy identification of chemical shifts. Entries with asterisks denote resonances where the assignment may possibly be inverted. [b] See footnote *b*, Table I. [c] Spectrum recorded at 15.1 MHz using the Fourier transform technique.[13] Chloroform was used as solvent and internal reference. [d] Spectrum recorded at 15.1 MHz using the Fourier transform technique.[13] Aqueous sodium bicarbonate solution (3%) was the solvent.

The assignment of the five carbonyl carbon frequencies (C-8, C-10, C-15, C-16, and C-18) was greatly facilitated by the examination of the cmr spectrum of the antibiotic labeled with $CH_3{}^{13}COONa$. Trown and coworkers have established the locus of acetate incorporation into cephalosporin C using $CH_3{}^{14}COONa$ and conventional radiotracer techniques.[8] Assuming that the acetate is incorporated as a unit analogously to the ^{14}C-labeled material, we have compared the cmr spectrum of the $CH_3{}^{13}COONa$-labeled antibiotic with the natural abundance spectrum and were able to assign the resonances at 14.1 and 14.6 ppm to C-15 and C-18, respectively. The data for the model systems, the shifts reported by others,[13,15] and the ^{13}C labeling results provided the assignment of the chemical shift at 12.0 ppm to C-10. The choice between the peaks at 20.6 and 24.3 ppm for C-8 and C-16 was still open; however, by utilizing the known pH dependence of the carboxyl shift (*cf.* ref 11), this uncertainty was resolved (Table II).



The use of $^{13}CH_3COONa$ in the fermentation of cephalosporin C resulted in labeling of the antibiotic in C-11, C-12, C-13, C-14, and C-19 (Table I). Of particular importance is the distribution of the label in C-11, C-12, and C-13. The amount of incorporation in these carbons appears to be about one-half of the incorporation at C-14.[16] This amount corresponds to the amount of the label dispersion one could expect to arise from cycling of the tricarboxylic acids involved in the formation of α-aminoadipic acid *via* the Krebs cycle.[17] This result clearly demonstrates the great utility of cmr spectroscopy in the study of biosynthetic pathways beyond that already shown by Tanabe and coworkers.[2]

Acknowledgments. The assistance of Mr. R. L. Pieper in the fermentation of cephalosporin C and in the pulse-labeling experiments is gratefully acknowledged. The synthesis of *dl*-α-aminoadipic acid ethylamide and the isolation of crystalline cephalosporin C–Na salt were expertly carried out by Mrs. Nancy

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De LaHiguera and Mr. L. L. Huckstep. We thank Dr. L. L. Hatfield for the sample of IV and Mr. W. Conover, of Indiana University, Bloomington, Ind., for recording the spectra of IV and V.

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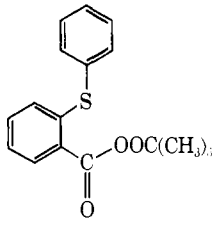
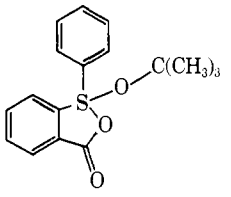
Sulfuranes. I. A Stable Tetracoordinate Tetracovalent Sulfur Compound in Solution

Sir:

For several years interest in our laboratory[1] in compounds of sulfur involving an expansion of the valence octet has centered about studies of neighboring group participation in the decomposition of peresters related to **1**. Compound **2** was postulated as a possible intermediate leading to some of the products observed in the decomposition of **1**. Numerous literature citations might be advanced in support of the postulated intermediacy of tetracovalent sulfur compound **2**. Indirect evidence for similar intermediates has been seen in kinetic,[2] spectroscopic,[3] and product[4] studies in several laboratories. In the special case of halide ligands both liquid[5] and crystalline[6,7] tetracoordinate sulfur compounds have been characterized. The geometry of sulfur tetrafluoride and its analogs[5] seems well established. The crystal structure of the unstable adduct of chlorine to 4,4′-dichlorodiphenyl sulfide was found[7] to involve approximately trigonal-bipyramid geometry about sulfur, with the *p*-chlorophenyl groups and an unshared pair of electrons occupying the equatorial plane and the chlorine atoms occupying apical positions. In contrast, the 1:1 adduct of thiophane with bromine has quite a different type structure with a tricoordinate sulfur atom.[8]

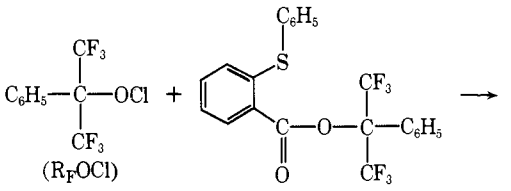


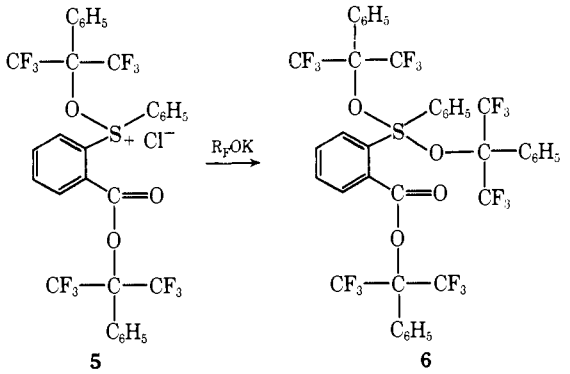
1



2

Dialkoxydialkyl- and hydroxyalkoxydialkylsulfuranes[9-12] have been suggested as intermediates in displacement reactions of alkoxysulfonium salts. On the other hand, stereochemical results of the base-catalyzed hydrolyses of *cis*- and *trans*-1-ethoxy-3-methylthietanium hexachloroantimonates have led to the suggestion[11] that in this case, in contrast to the situation for the analogous phosphetanium salt, intermediates with two alkoxy groups attached to sulfur have a very short lifetime relative to the half-life for pseudorotation, or perhaps represent transition states for a direct SN2 displacement on sulfur.





Hexafluoro-2-phenyl-2-propanol (R_FOH) is converted to hypochlorite **3** (R_FOCl) by chlorination of the alkoxide (R_FOK) in anhydrous $CFCl_3$ at −78°.[13] Reaction of **3** with sulfide **4** in CH_2Cl_2 at −78° results in formation of alkoxysulfonium chloride **5**. Sulfurane **6** is formed upon addition of the alkoxide, R_FOK, in ether to **5** at −78°. A more convenient synthesis of **6** involves treatment of a mixture of **4** and R_FOK, in ether at −78°, with chlorine.

We have obtained nmr evidence for the covalent nature of the S–O bonds in **5** and **6** by using the hexafluorocumyloxy ligands as ^{19}F nmr probes. The six fluorines of compound **4** give a single nmr peak, which broadens markedly at low temperatures (−70°). Figure 1 shows low-temperature spectra for **5** and **6**. The

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(12) This nomenclature, based on the name "sulfurane" for the hypothetical SH_4 molecule, is loosely analogous to that used for the phosphorus analogs, the oxyphosphoranes. See F. Ramirez, *ibid.*, 1, 168 (1968). The term "sulfoxal" has also been suggested[10] to describe compounds of structure $R_2S(OR)_2$, such as **6**.

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