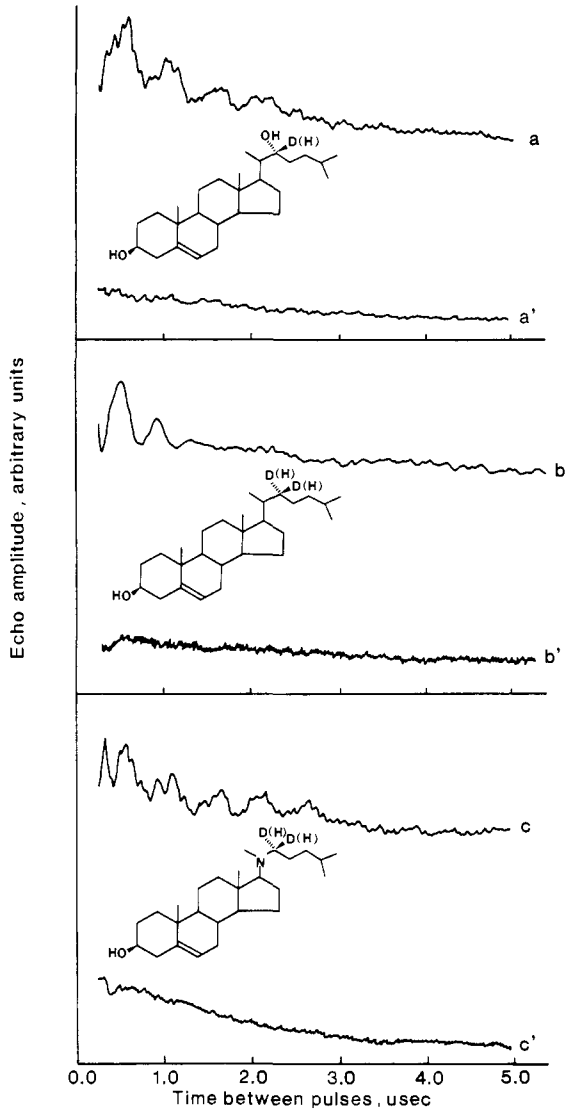

Figure 1. Three-pulse ESE modulation ($\pi/2$–τ–$\pi/2$–T–$\pi/2$–τ... echo) for ferric P-450$_{scc}$–substrate complexes, recorded at g = 2.2 (9.2 GHz), 6–7 K, τ = 250 ns. Spectra are displaced for clarity. Substrates: (a) 22(R)-hydroxycholesterol-*22-d*; (a′) 22(R)-hydroxycholesterol; (b) cholesterol-*22,22-d*$_2$; (b′) cholesterol; (c) 20-azacholesterol-*22,22-d*$_2$; (c′) 20-azacholesterol. Concentrations: P-450, 400–700 μM; steroids, 0.5–1 μM (1.5-fold over heme); in GPED (20% glycerol, 1 mM ethylenediaminetetraacetic acid disodium salt, 0.1 mM dithiothreitol, and 0.1 M potassium phosphate, pH 7.4).

estingly, we find no sign of ^{2}H modulation for the "unnatural" isomer 22(S)-hydroxycholesterol-*22-d*; this suggests strongly that the deuteron in this complex does not couple significantly to the electron spin and is thus presumably more than 6 Å from the heme.

In the cases of cholesterol-*22,22-d*$_2$ and 20-azacholesterol-*22,22-d*$_2$, slightly different modulation patterns are observed. Detailed analyses of these spectra are more difficult given the presence of two I = 1 nuclei and are presently under way. Clearly, however, both cholesterol and the 20-azacholesterol bind in close proximity to the heme.

These experiments demonstrate the potential of electron spin echo spectroscopy to probe the structural aspects of substrate binding to paramagnetic enzymes. The method may be of particular interest with membrane-bound systems (such as P-450$_{scc}$), which are not presently amenable to crystallographic investigations. By examining a more complete set of deuterated steroids it should be possible to approximate the relative position of a substrate with respect to the catalytic site of P-450$_{scc}$ under nonperturbing conditions; these studies are in progress.

Similar deductions for other enzymes have been derived from NMR relaxation measurements on substrate molecules in equilibrium with paramagnetic E·S complexes.[12] The NMR method requires the evaluation of multiple spin relaxation times and chemical exchange rate constants but can be done at room temperature. The ESEEM method requires no such additional information but usually must be carried out at very low temperatures. Both methods give interpretable data only when dipolar, Fermi contact and quadrupolar contributions can be estimated properly.

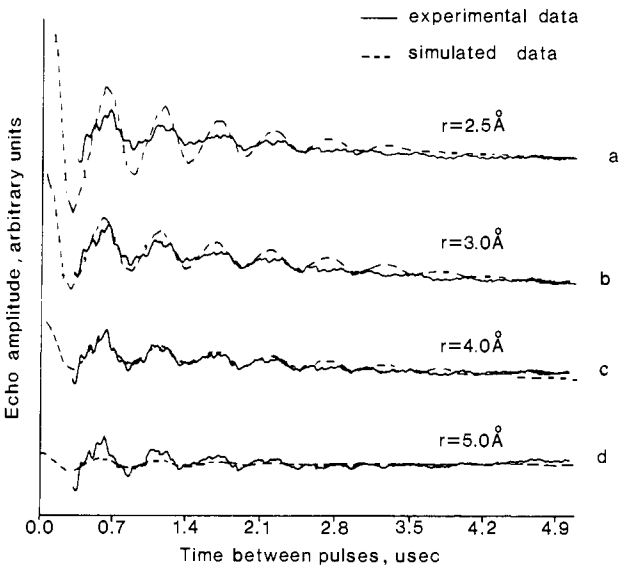

Figure 2. Comparison of experimental and simulated three-pulse ESE deuterium modulation for the data of Figure 1a. Simulations were according to the method of Suryanarayana et al.,[10] using a 0.1 isotropic hyperfine term and a quadrupolar term of 0.05 MHz.[11]

Acknowledgment. This work was supported by grants from the USDA (No. 5901-0410-9-0226-0) and the NIH (No. GM28358). S.E.G. acknowledges an NIH postdoctoral fellowship.

(12) For calculations on pyruvate kinase E·S·Mn complexes, see, e.g.: Mildvan, A. S.; Sloan, D. L.; Fung, C. H.; Gupta, R. K.; Melamud, E. *J. Biol. Chem.* **1976**, *251*, 2431.

Investigations of the Biosynthesis of Furanomycin. Unexpected Derivation from Acetate and Propionate

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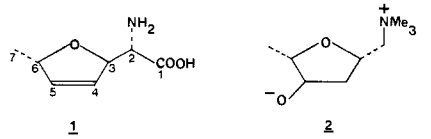
In 1967, Katagiri et al. reported the isolation of the novel antibiotic furanomycin (**1**) from *Streptomyces threomyceticus* (ATCC 15795).[1] The compound was found to be a competitive antagonist of L-isoleucine and to inhibit the growth of T-even coliphage. Furanomycin was synthesized in 1980 by Joullié and co-workers who also revised the stereochemistry to that shown in **1**.[2] The structure of furanomycin bears some resemblance to



1 **2**

(1) Katagiri, K.; Tori, K.; Kimura, Y.; Yoshida, T.; Nagasaki, T.; Minato, H. *J. Med. Chem.* **1967**, *10*, 1149.
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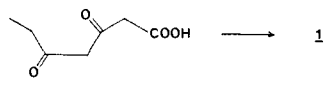
Table I. Precursor Incorporation Experiments with *S. Threomyceticus*

experiment no.	precursor ($^3H/^{14}C$)	% incorporation ($^3H/^{14}C$)	location of label
1	[U-^{14}C]-L-glutamic acid	0.03	
2	sodium [U-^{14}C]pyruvate	0.04	
3	sodium [1-^{14}C]acetate	1.0	
4	sodium (1-^{13}C)acetate	ca. 1.6	C-1, C-3
5	sodium (2-^{13}C)acetate	ca. 1.5	C-2, C-4
6	sodium (1,2-^{13}C)acetate	ca. 1.2	C-1, C-2 ($^1J_{CC}$ = 54 Hz) C-3, C-4 ($^1J_{CC}$ = 40 Hz)
7	sodium (1-^{13}C)propionate	ca. 7.3	C-5
8	DL-sodium [U-^{14}C]lactate	0.04	
9	sodium [1-^{14}C,2(*R*,*S*)-^{3}H]propionate (5.42)	6.0 (2.43)	

that of the agaric toxin muscarine (**2**) whose biosynthesis apparently proceeds from pyruvate and glutamate.[3] Nevertheless, experiments will now be outlined which prove that the biosynthetic pathways to **1** and **2** are unrelated.

Streptomyces threomyceticus (ATCC 15795) was cultivated according to the procedure of Katagiri et al.[1] and precursors were added after 72 h. Because of the structural similarity between furanomycin and muscarine, [U-^{14}C]-L-glutamate and sodium [U-^{14}C]pyruvate were initially evaluated as precursors. The results of these experiments (Table I, experiments 1 and 2) suggested that neither of these compounds was a direct precursor of furanomycin. On the other hand, administration of sodium [1-^{14}C]acetate to cultures of *S. threomyceticus* yielded antibiotic whose radioactivity corresponded to an incorporation figure of 1% (Table I, experiment 3). The specific incorporation of acetate was then demonstrated by administration of sodium (1-^{13}C)acetate to *S. threomyceticus*. This experiment produced furanomycin whose proton noise-decoupled NMR spectrum[4] revealed substantial enrichment at C-1 and C-3 of the antibiotic (Table I, experiment 4). This observation indicated that two acetate units are incorporated into furanomycin, a fact that was confirmed by an incorporation experiment with sodium (2-^{13}C)acetate. As expected, the furanomycin derived from this form of labeled acetate exhibited ^{13}C enrichment at C-2 and C-4 (Table I, experiment 5). A final confirmation of the incorporation of two intact acetate units into **1** was obtained by administration of sodium (1,2-^{13}C)acetate to the producing organism (experiment 6).

The experiments with ^{13}C-labeled acetate yielded furanomycin that exhibited no enrichment in C-5 to C-7. A logical precursor of this segment of the antibiotic was deemed to be propionate, and indeed, administration of sodium (1-^{13}C)propionate to *S. threomyceticus* yielded **1** exhibiting a high degree of enrichment at C-5 (experiment 7). We therefore conclude that furanomycin is derived from two acetate units and one propionate unit, with the latter serving as the starter unit (see eq 1).



⟶ **1** (1)

The incorporation of propionate into **1** involves the introduction of oxygen at C-2 of propionate. A priori, this could occur either before or after the assembly of a putative seven-carbon diketo acid. If C-2 of propionate were oxidized before the assembly process, then lactic acid would be a likely intermediate in furanomycin biosynthesis. This possibility was evaluated by an incorporation experiment with DL-sodium [U-^{14}C]lactate. The low incorporation figure that was observed (Table I, experiment 8) makes it unlikely that propionate is hydroxylated to lactate prior to the assembly process. Some additional insight into the mechanism of oxidation of C-2 of propionate was obtained by utilizing sodium 2(*R*,*S*)-[2-^{3}H]propionate as a precursor. The tritiated acid was prepared by generation of the anion of *n*-butyl propionate with LDA[5] followed by quenching with [^{3}H]trifluoroacetic acid. The resulting tritiated ester was then converted to its sodium salt and mixed with sodium [1-^{14}C]propionate. Administration of the doubly labeled propionate to *S. threomyceticus* yielded furanomycin that retained 45% of the tritium label (experiment 9). This result rules out the possible formation of a keto function at C-2 of propionate during the biosynthesis, and it indicates that the formation of the ether linkage in furanomycin involves the loss of one hydrogen atom from the prochiral center at C-2 of propionate. Future experiments will examine the stereochemistry of formation of the ether linkage and the origin of the oxygen atom as well as the mechanism of nitrogen introduction at C-2 of the furanomycin skeleton.

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Transmission of Magnetic Interactions in a Molecular Metal Oxide Cluster

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Molecular metal oxides and alkoxides have recently been considered to be useful models of condensed-phase metal oxides[1,2] that may provide valuable information about catalytic chemistry on bulk oxide surfaces and about the interaction of metal catalysts with such surfaces.[3,4]

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