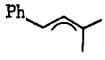
Table I

Allyl group of π-(allyl)Fe(CO)$_4^+BF_4^-$	Nucleophile	Product (% yield)[a]
	PPh_3	$(CH_2{=}CHCH_2PPh_3)Fe(CO)_4^+BF_4^-$
	Pyridine	$(CH_2{=}CHCH_2NC_5H_5)Fe(CO)_4^+BF_4^-$ (85)
	$CH_2{=}C(OAc)CH_3$[b]	$CH_2{=}CHCH_2CH_2COCH_3$ (8)
	$CH_3COCH_2CO_2CH_3$[c]	$CH_2{=}CHCH_2CH(CO_2CH_3)COCH_3$ (28)
	PPh_3	*cis*-$CH_3CH{=}CHCH_2PPh_3^+BF_4^-$ (74)
	PPh_3	*cis*-$CH_3CH{=}CHCH(CH_3)PPh_3^+BF_4^-$ (50)
	PPh_3	$(CH_3)_2C{=}CHCH_2PPh_3^+BF_4^-$ (74)[e]
	Pyridine	$(CH_3)_2C{=}CHCH_2NC_5H_5^+BF_4^-$ (80)[e]
	$CH_3COCHCO_2CH_3$[d]	$(CH_3)_2C{=}CHCH_2CH_2COCH_3$ (68)
		$CH_2{=}CHC(CH_3)_2CH_2COCH_3$ (17)
	$(CH_3CH_2)_2NH$	$(CH_3)_2C{=}CHCH_2N(CH_2CH_3)_2$ (28)
	$PhCH(NH_2)CH_3$	$(CH_3)_2C{=}CHCH_2NHCH(CH_3)Ph$ (28)
		$CH_2{=}CHC(CH_3)_2NHCH(CH_3)Ph$ (13)
	PPh_3	$C_6H_5CH(PPh_3)CH{=}C(CH_3)_2^+BF_4^-$

[a] Isolated. [b] Conditions: refluxed for 40 min at 90°. [c] Conditions: refluxed in acetone for 4 hr. [d] Products isolated after saponification and decarboxylation. [e] Use of stereospecifically labeled allyl cation complex leads to stereospecifically labeled isophenyl adduct.

The availability of π-allyliron tetracarbonyl cation[5] allowed us to make certain observations concerning the probable mechanism of these transformations. This species gave rise to reasonably stable olefin–iron tetracarbonyl complexes after treatment with, for example, triphenylphosphine, pyridine, or the anion of methylacetoacetate. Particularly in the case of the phosphonium and pyridinium salts, these novel organometallic species were readily isolable.[7] Some evidence (nmr) for similar intermediates was obtained in the case of species with disubstituted double bonds, but these were much less stable than the monosubstituted olefin complexes derived from the allyl species, decomposing rapidly to the uncomplexed organic moiety and (at least partially) $Fe_3(CO)_{12}$[8] at room temperature. These observations indicate that the initial attack of the nucleophile occurs on the ligand, with subsequent decomposition of the iron(0) complexes so formed, the decomposition being accelerated by increasing substitution of the double bond. As seen in Table I, nucleophilic attack in general occurs preferentially at the unsubstituted end of the coordinated allyl group.

The procedure for these alkylations is very simple. Iron salt is suspended in THF or ether and the appropriate nucleophile is added. Usually, with reasonably nucleophilic species, reaction is instantaneous as indicated by solution of the insoluble salt. Work-up is then carried out in the normal manner.

We are continuing to investigate the scope and synthetic utility of this novel reaction.

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(5) This species was prepared by treating π-allyliron tricarbonyl iodide[6] with $AgBF_4$ in the presence of CO, followed by precipitation of the cation by addition of ether.

(6) R. B. King, "Organometallic Synthesis," Vol. 1, Wiley, New York, N. Y., 1965, p 176.

(7) The physical properties of allylpyridiniumiron tetracarbonyl tetrafluoborate are: ir (CH_2Cl_2) 2100, 2032, 2012, 1996 cm^{-1} (ν_{CO}); nmr (acetone-d_6) δ 9.28 (2 H, d), 8.71 (1 H, t), and 8.22 (2 H, t), pyridine hydrogens; 5.60 (1 H, dd, J = 3.4, 13 Hz) and 4.64 (1 H, dd, J = 13, 11.5 Hz), $-CH_2^+N\ll^+$; 3.85 (1 H, mult), $-CH{=}$; and 3.03 (1 H, dd, J = 2, 12 Hz) and 2.87 (1 H, dd, J = 2, 8.5 Hz), $H_2C{=}$. *Anal.* Calcd for $C_{12}H_{10}NFeO_4BF_4$: C, 38.45; H, 2.69; N, 3.75; Fe, 14.90. Found: C, 38.42; H, 2.74; N, 3.78; Fe, 14.67. The phosphonium compounds are similar. In particular the shift of the ν_{CO} bonds to longer wavelength relative to the allyl cation (2123, 2062 cm^{-1}) indicates a reduction of the charge on the metal, and the high chemical shift of the olefinic protons indicates complexation of the olefinic moiety.

(8) (a) G. Cardaci and V. Narciso, *J. Chem. Soc., Dalton Trans.*, 2289 (1972); (b) K. Von Gustorf, M. C. Henry, and D. J. McAdoo, *Justus Liebigs Ann. Chem.*, 707, 190 (1967).

Carbon-13 Magnetic Resonance Spectroscopy and the Biosynthesis of Streptovaricin[1,2]

Sir:

The streptovaricins, rifamycins, and their derivatives have aroused considerable recent interest due to their inhibition of RNA dependent DNA polymerase (reverse transcriptase) from RNA tumor viruses, their very potent inhibition of DNA dependent RNA polymerase from *E. coli*, and their general antibacterial and antiviral properties, especially against mycobacteria.[2a,b,3]

In spite of the biological interest noted above and their remarkable ansa structures, the biosynthesis of these compounds and the related antibiotics tolypomycin and geldanamycin remains unreported. From their structures (*e.g.*, that of streptovaricin D, **1** in Figure 1) propionate and acetate seem the likely candidates as precursors for the aliphatic bridge and,

(1) Paper II in the series "Carbon-13 as a Biosynthetic Tool" [Paper I: W. M. J. Knöll, R. J. Huxtable, and K. L. Rinehart, Jr., *J. Amer. Chem. Soc.*, **95**, 2703 (1973)] and Paper X in the series "Chemistry of the Streptovaricins" [Paper IX: A. H.-J. Wang, I. C. Paul, K. L. Rinehart, Jr., and F. J. Antosz, *ibid.*, **93**, 6275 (1971)].

(2) (a) Presented at the Symposium on Ansamycin Antibiotics and their Biological Activities, Abstracts, 165th National Meeting of the American Chemical Society, Dallas, Texas, April 8–13, 1973, MEDI-2. Other papers in the symposium: (b) P. Sensi, *ibid.*, MEDI-1; (c) R. C. Gallo, *ibid.*, MEDI-3.

(3) Recent reviews: (a) K. L. Rinehart, Jr., *Accounts Chem. Res.*, **5**, 57 (1972); (b) W. Wehrli and M. Staehelin, *Bacteriol. Rev.*, **35**, 290 (1971).

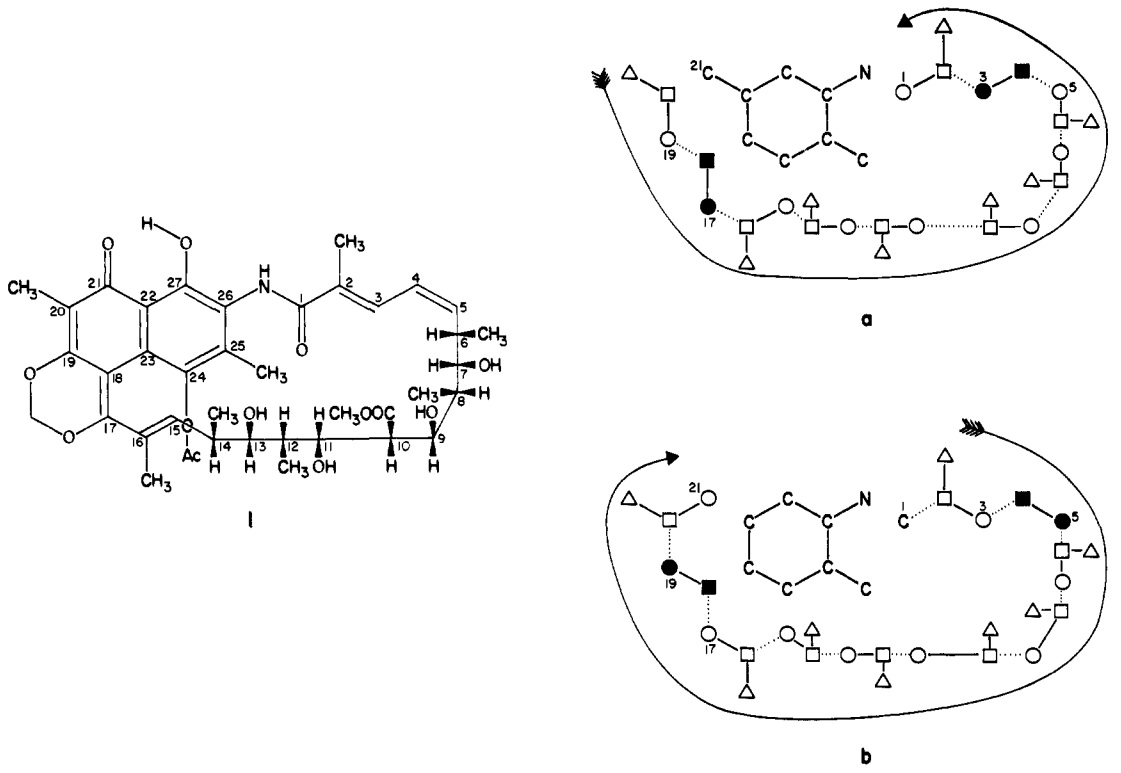

Figure 1. Streptovaricin D (**1**) and two potential biosynthetic pathways for its formation: (a) amide-head path, (b) amide-tail path.

Table I. Incorporation of Labeled Precursors into Streptovaricin D (SvD)

	[*methyl*-^{14}C]-Methionine	Sodium [*carboxy*-^{14}C]-malonate	Sodium [*carboxy*-^{14}C]-propionate	Sodium [*carboxy*-^{13}C]-propionate
Precursor added				
Amount	0.071 mg	0.074 mg	0.037 mg	3.0 g
Label	42.6 mCi/mmol	40.0 mCi/mol	54.9 mCi/mmol	90% ^{13}C
SvD isolated				
Amount				30 mg
Label				15% ^{13}C[a]
% incorp	0.11	0.13	0.032	0.17
Dilution				6.0

[a] Estimated from mass spectra of enriched SvD, assuming equal labeling at eight carbons.

perhaps, for the aromatic rings as well. This hypothesis was reviewed earlier,[3a,4] where it was noted that propionate can be assembled in the side chain in two ways: an amide-head direction (Figure 1, path a), with the amide carbonyl derived from the carboxyl group of propionate, or an amide-tail direction (path b), with the amide carbonyl derived from a one-carbon unit. Although path a is the more obvious, path b has been reported for the biosynthesis of rifamycin.[4]

Our own experiments with streptovaricin (Table I) demonstrated [*carboxy*-^{14}C]propionate to be well incorporated, as was [*methyl*-^{14}C]methionine, the expected precursor of the methoxy and methylenedioxy carbons, and perhaps of one or more C-methyl groups. The question of amide-head *vs.* amide-tail remained.

To settle this question the key carbon atoms are the amide carbon itself (C-1) and C-3 and C-5 of the dienamide unit, other carbon atoms (*e.g.*, C-7, C-9, C-11, C-13, C-15) being labeled by either path a or path b. In path a the amide carbonyl (C-1) and C-5 (but not C-3) would be labeled by propionate carboxyl, while in path b C-3 (but not C-1 and C-5) would be labeled by the same substrates. Carbon magnetic resonance employing carbon-13 label provides a convenient procedure for deciding the case.

Sodium [*carboxy*-^{13}C]propionate was added (Table I) after 2 days to 4.0 l. of fermentation broth inoculated with *Streptomyces spectabilis* as described earlier.[5] After a total of 5 days growth the streptovaricin produced was isolated, and the components were separated by the usual procedure.[6]

Isotope ratio mass spectra (flat top peaks) of the labeled streptovaricin D (**1**) indicated approximately 53% unlabeled streptovaricin D, 14% monolabeled, 12% dilabeled, 15% trilabeled, and 7% tetralabeled. (This corresponds to an average label of 15% (Table I) at each of the eight carbon atoms labeled (*vide*

(4) J. W. Corcoran and M. Chick in "Biosynthesis of Antibiotics," Vol. I, J. F. Snell, Ed., Academic Press, New York, N. Y., 1966, p 159.

(5) A. Dietz, C. De Boer, R. M. Smith, P. Siminoff, G. A. Boyack, and G. B. Whitfield, U. S. Patent 3,116,202 (1963); *Chem. Abstr.*, 60, 9871*h* (1964).

(6) K. L. Rinehart, Jr., P. K. Martin, and C. E. Coverdale, *J. Amer. Chem. Soc.*, 88, 3149 (1966).

sequitor).) The carbon magnetic resonance spectrum of unlabeled streptovaricin D (**1**) showed peaks for the expected 40 carbon atoms. Relevant carbon atom signals were assigned (Table II) with the help of the

Table II. Important Carbon Magnetic Resonance Peaks for Streptovaricin D (**1**)

Carbon atom[a]	δc[b]	Rel enrichment[c]
C-1	169.4	0.7
C-3	134.7	0.0
C-5	144.1	1.3
C-7	83.6	0.8
C-9	77.6	1.1
C-11	73.4[d]	1.0
C-13	70.4[d]	0.8
C-15	153.6	1.1
C-17	169.0	0.0
C-19	159.6	1.3
C-21	188.7	0.0

[a] Numbering for **1** is shown in Figure 1. [b] Ppm from TMS; CD_2Cl_2 = 53.80. [c] Calculated by measuring peak heights in the spectrum of enriched streptovaricin D relative to the height (arbitrarily assigned the value 1.00) of the peak at 153.6 ppm (the tallest of these peaks in the unenriched spectrum), then dividing those relative heights by the relative heights of the same peaks (calculated the same way, from an assigned value 1.00 for the peak at 153.6 ppm) in the natural abundance spectrum. The relative enrichments, which of necessity are rough approximations, have been normalized so their sum is 8.0. [d] May be interchanged.

carbon magnetic resonance spectra of other streptovaricins; of standard chemical shift data;[7] and of complete, off resonance, and specific proton[8] decoupling.[9]

The carbon magnetic resonance spectrum of ^{13}C-labeled streptovaricin D showed eight clearly enriched peaks of similar intensity for C-1, C-5, C-7, C-9, C-11, C-13, C-15, and C-19. Most important, the present data establish that the amide-head pathway (path a) is that followed, since the amide carbon (C-1) and C-5 are labeled by propionate carboxyl but C-3 is not. That one of the ring carbons (C-19) is labeled by propionate carboxyl implies a continuous sequence of propionate and acetate units leading from C-20 through C-1. We assume acetate (or malonate) as the origin of C-3 and C-4 and of C-17 and C-18.

The quinone carbonyl (C-21) is unlabeled by propionate, as are C-26 and C-24, the carbons adjacent to the remaining methyl group at C-25.[10] The lack of a methyl at the carbon in rifamycin[3] corresponding to C-25 suggests that our C-25 methyl group comes from methionine. The origin of the benzenoid aromatic ring and the quinone carbonyl carbon (C-21 through C-27) remains to be established. Experiments along these lines are in progress.

Acknowledgments. This work was supported by Public Health Service Grants AI 01278 and AI 04769 from the National Institute of Allergy and Infectious Diseases and in part by Contract NIH-NCI-C-72-3208 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare. We thank Mr. Robert Thrift for the ^{13}C nmr spectra, obtained on a spectrometer purchased with an instrumentation grant from the National Science Foundation, Mr. Joseph Wrona for the isotope ratio mass spectra, and Dr. Robert F. Nystrom for assistance with the synthesis of ^{13}C-labeled propionate.

Supplementary Material Available. A more detailed description of assigned chemical shifts will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 20× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St,, N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JACS-73-5793.

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Collision-Induced Negative Ion Mass Spectrometry[1]

Sir:

We have previously shown that certain classes of organic compounds will accept an electron to produce molecular anions which may then undergo characteristic decompositions.[2,3] It has been demonstrated[4-6] that molecular anions may be formed by secondary electron capture under these conditions,[7] and it follows that our spectra may be produced by the decomposition of molecular anions with thermal or near thermal energies. This has been substantiated for the case of the 2-aryl-1,3-oxathianes.[8]

There are many molecular anions which do not decompose, and there are some functional groups which show no fragmentation in the negative mode. It is

(7) (a) L. F. Johnson and W. C. Jankowski, "Carbon-13 NMR Spectra," Wiley-Interscience, New York, N. Y., 1972; (b) J. B. Stothers, "Carbon-13 NMR Spectroscopy," Academic Press, New York, N. Y., 1972; (c) G. C. Levy and G. L. Nelson, "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists," Wiley-Interscience, New York, N. Y., 1972.

(8) K. L. Rinehart, Jr., M. L. Maheshwari, F. J. Antosz, H. H. Mathur, K. Sasaki, and R. J. Schacht, *J. Amer. Chem. Soc.*, **93**, 6274 (1971).

(9) A more detailed description of the methods used to assign chemical shifts to individual carbon atoms will be published in the Proceedings of the First International Conference on Stable Isotopes in Chemistry, Biology and Medicine, Argonne National Laboratory, May 9–11, 1973, USAEC Publication, CONF-730525, and in the microfilm edition of this journal. See paragraph at end of paper regarding supplementary material.

(10) After our presentation of these streptovaricin biosynthesis results,[2a] we were informed by Dr. P. Sensi, Gruppo Lepetit, Milan, Italy, that workers in his laboratory had reached similar conclusions on the biosynthesis of rifamycin, employing carbon-13 label, again contradicting the earlier (unpublished) results.[4]

(1) This investigation was supported by Grant C67/16756 from the Australian Research Grants Committee.

(2) J. H. Bowie, A. C. Ho, and A. Fry, *J. Chem. Soc. B*, 530 (1971).

(3) For a recent review see J. H. Bowie, *Mass Spectrom.*, **2**, 137 (1973).

(4) G. Jacobs and A. Henglein, *Advan. Mass Spectrom.*, 289 (1966).

(5) J. C. J. Thynne, *Chem. Commun.*, 1075 (1968); P. W. Harland, K. A. C. MacNeil, and J. C. J. Thynne, *Dyn. Mass Spectrom.*, **1**, 122 (1970).

(6) T. McAllister, *J. Chem. Soc., Chem. Commun.*, 245 (1972).

(7) Conditions used: Hitachi Perkin-Elmer R.M.U. 7D instrument, source pressures 2–5 × 10^{-7} Torr, electron beam energy 70 eV.

(8) J. H. Bowie and A. C. Ho, *Aust. J. Chem.*, in press.