Table I. ^{1}H NMR Chemical Shift Data in SO_2

Compound	α-CH	η^5-C_5H_5	SiMe	Ph
3	δ 4.89	δ 4.32	δ −0.1	δ ~7.2
4	δ 5.27	δ 4.54	δ 0.07	δ ~7.2

carbon bonds are normally highly stereospecific, occurring with inversion of configuration at the α-carbon atom with the compounds η^5-$C_5H_5Fe(CO)_2R$ (R = *threo*-$CHDCHDCMe_3$,[4] *threo*-CHDCHDPh[5]), stereospecifically, at least, with $(+)_{546}$-η^5-$C_5H_5Fe(CO)_2CHMePh$,[6] and with retention of configuration at iron with η^5-$C_5H_5FeCOPPh_3CH_2CO$(mentholate).[7] Therefore the reaction of **1** with SO_2 was investigated in detail in an attempt to elucidate the mechanism of the epimerization reaction, which does not occur in a number of organic solvents in the absence of SO_2.

In accord with established procedures,[2,3] **1** was refluxed in SO_2 for 5 hr, the solvent was allowed to evaporate and the solid residue was extracted with methylene chloride and eluted on an alumina column. The product contained no SO_2 but was rather a 40:60 mixture of **1** and **2**. A similar result was obtained by bubbling SO_2 through a solution of **1** in methylene chloride, although slight paramagnetism of the solution precluded following the course of the reaction using NMR spectroscopy.

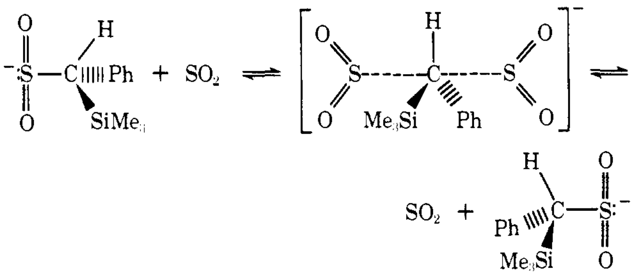
The reactions of **1** (and **2**) with SO_2 were, however, followed successfully using ^{1}H NMR spectroscopy in SO_2 solvent. Both **1** and **2** reacted essentially completely in neat SO_2 at 293 K, **2** faster than **1**, to form the corresponding *S*-sulfinates, **3** and **4**, respectively. Although the latter compounds lost SO_2 both in the solid state and in organic solvents too rapidly to be characterized properly, comparison of their NMR parameters (Table I) with those of similar complexes[2,3] permitted their identification. (Presumably **3** and **4** have the *RR*-*SS* and *RS*-*SR* configurations, respectively[1]). On standing in SO_2 for 1 hr, **3** and **4** equilibrated with each other such that, at equilibrium, [**3**]:[**4**] ≈ 3:1; these results can be summarized as follows:

$$1 + SO_2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} 3 \qquad k_2 > k_1$$
$$3 \underset{k_4}{\overset{k_3}{\rightleftharpoons}} 4$$
$$2 + SO_2 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} 4 \qquad k_4 > k_3$$

If the reaction of **1** with liquid SO_2 were halted after the conversion to **3** was completed but before significant amounts of **4** were formed, solid **3** could be obtained by quickly removing the SO_2 at reduced pressure. While extrusion of SO_2 from solid **3** *in vacuo* at 328 K yielded pure **1**, loss of SO_2 from **3** in CD_2Cl_2 solution at 283 K in a sealed NMR tube initially yielded **2** faster than **1**, although **1** dominated as equilibrium was approached. In the latter reaction, no **4** could be detected. Also relevant are the observations that SO_2 did not induce epimerization of a solution of **1** in the nonpolar petroleum ether, that rapid removal by pumping of the extruded SO_2 during the decomposition of **3** at room temperature in methylene chloride, chloroform, or benzene resulted in [**2**]:[**1**] ratios as high as 4:1, but that heating a solution of **3** during extrusion or preventing the SO_2 from escaping until the system had approached equilibrium resulted in [**1**]:[**2**] ratios of 3:1 or higher.

The facile formation of the thermodynamically less stable **2** on decomposition of **3** presumably occurs via **4**, and must occur because the **3** → **4** → **2** sequence is favored kinetically over the **3** → **1** path; certainly the free energy of activation for the conversion of **1** to **3** is greater than that for **2** → **4**.[8] Thus, the rate of formation of **1** becomes competitive when sufficient heat is supplied to overcome the **3** → **1** activation barrier, while retention of SO_2 in solution allows the system to eventually equilibrate to the thermodynamically preferred **1**. On the other hand, cooling the system would make the **3** → **4** → **2** conversion kinetically more viable, while rapid removal of SO_2 from a solution containing predominantly **2** would prevent equilibration to **1**.

The hypothesis requires a mechanism for the interconversion of **3** and **4** which also involves epimerization at one of the chiral centers, a reaction which does not occur significantly with the compounds η^5-$C_5H_5Fe(CO)_2R$ (R = *threo*-$CHDCHDCMe_3$,[4] *threo*-CHDCHDPh,[5] and CHMePh[6]). We suggest that, because of greater strain, **3** and **4** dissociate to some extent in polar solvents to give the ions $[\eta^5$-$C_5H_5FeCOP(OPh)_3(solvent)]^+$ and $[SO_2CHPhSiMe_3]^-$. The latter would then be susceptible to electrophilic attack by a second molecule of SO_2, resulting in a Walden-like inversion, i.e.



Ions such as the above are believed to be intermediates in the reactions of metal-alkyl complexes with SO_2 to give sulfinates.[9] The mechanism is also consistent with the observations that SO_2 does not induce epimerization of **1** in a poorly ionizing solvent such as petroleum ether. Experiments are now underway to determine if the epimerization does indeed take place at the α-carbon atom.

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References and Notes

(1) K. Stanley and M. C. Baird, *J. Am. Chem. Soc.*, preceding paper in this issue.
(2) M. Graziani and A. Wojcicki, *Inorg. Chim. Acta*, **4**, 347 (1970).
(3) S. R. Su and A. Wojcicki, *J. Organomet. Chem.*, **27**, 231 (1971).
(4) P. L. Bock, D. J. Boschetto, J. R. Rasmussen, J. P. Demers, and G. M. Whitesides, *J. Am. Chem. Soc.*, **96**, 2814 (1974).
(5) D. Slack and M. C. Baird, unpublished results.
(6) J. J. Alexander and A. Wojcicki, *Inorg. Chim. Acta*, **5**, 655 (1971).
(7) T. C. Flood and D. L. Miles, *J. Am. Chem. Soc.*, **95**, 6460 (1973).
(8) Kinetic and equilibrium studies suggest that the difference in activation energies of the 3 → 1 and 4 → 2 conversions is about 2.8 kcal.
(9) A. Wojcicki, *Adv. Organomet. Chem.*, **12**, 31 (1974).

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Biosynthetic Studies Using ^{13}C Enriched Precursors on the 16-Membered Macrolide Antibiotic Leucomycin A_3

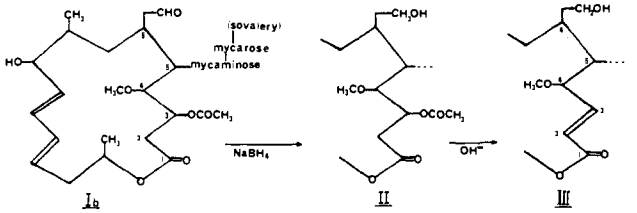
Sir:

According to previous reports[1] the aglycone carbons of the 16-membered macrolide antibiotic magnamycin B (Ia) are derived, as shown in Figure 1, from nine acetates, one propionate, and one methionine. As an application of a recent systematic ^{13}C NMR study of 16-membered macrolide antibiotics[2] the validity of these investigations was reexam-

Table I. Incorporation of ^{13}C-Labeled Precursors into Leucomycin A_3

Carbon atoms	Chemical shift δ (ppm) from $(CH_3)_4Si$	Relative intensity (%)[a, b]			
		[1-^{13}C] Acetate	[2-^{13}C] Acetate	[1-^{13}C] Propionate	[1-^{13}C] Butyrate
C-1	169.9	11.6			2.7
C-2	37.0		81.7		
C-3	71.6				
C-4[c]	84.9				
C-5[c]	77.5	19.9			100
C-6	28.8		9.0		
C-7	30.4		⟨5.0	100	
C-8	33.5		8.2		
C-9	73.1	100			4.1
C-10	127.6		100		
C-11	135.7	61.2			6.5
C-12	132.1		74.3		
C-13	132.6	60.3			4.9
C-14	40.9		81.5		
C-15	68.8	71.1			4.6
C-16	20.3		92.1		
C-17	42.4	4.8			
C-18	201.2		8.0		
C-19	14.7		3.1		
C-20	170.8	21.6			7.2
C-21	21.3		64.9		

[a] Relative intensity %; the highest peak of the spectrum, as a reference, is taken for 100 and the height of the other peaks is compared with it. [b] Under the experimental conditions the natural abundance peaks were not observed. [c] Biosynthetic evidence as well as spectral considerations indicate that the resonance assignment for C-4 and C-5 of Ib as suggested in our previous study[2] should be reversed. These signals appear in the spectrum of Ib at 77.5 and 84.9 ppm and were mistakenly attributed respectively to C-4 and C-5. Inspection of the ^{13}C NMR spectra of 18-dihydro-leucomycin A_3 II and 3-deacetoxy-2-dehydro-18-dihydro-leucomycin A_3 III reveals that the consequence of the deacetoxylation is to shield the 84.9 ppm signal and to deshield the 77.5 ppm resonance of Ib. The corresponding two signals of III appear at 82.9 and 82.3 ppm. This observation can be reconciled in the spectrum of Ib only with the 84.9 ppm signal representing C-4 and the 77.5 ppm signal C-5 in agreement with the proposed biosynthetic scheme.



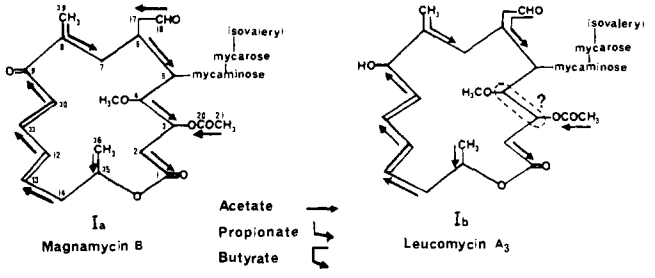


Figure 1. Biosynthesis of Magnamycin B (Ia) and Leucomycin A_3 (Ib).

Table II. The Change of the Relative Incorporation Ratio of [2-^{13}C] Acetate by the Addition of Cold Butyrate to the Medium

Relative intensity	[2-^{13}C] Acetate + cold butyrate	[2-^{13}C] Acetate
C-2/C-16	0.98	0.73
C-6/C-16	0–0.05	0.21
C-8/C-16	0.12	0.17
C-10/C-16	2.28	1.95
C-12/C-16	1.62	1.89
C-14/C-16	0.91	0.80
C-18/C-16	0.13	0.37
C-21/C-16	0.84	0.91

ined on leucomycin A_3 (Ib) which is the dihydro product of magnamycin B, Ia. We report in this communication that carbon-5, -6, -17, and -18 are derived from butyrate. Furthermore, carbons-3 and -4 do not arise from acetate as proposed previously, their origin being unknown at present time.

Streptomyces kitasatoensis No. 66-14-3, a strain producing leucomycins, was cultured in modified Waksman media (glucose 0.7%, peptone 0.5%, yeast extract 0.1%, meat extract 0.5%, NaCl 0.5%, and $CaCO_3$ 0.3% (pH 7.0)). The ^{13}C-precursors (0.1–0.2%, w/w), 90% enriched [1-^{13}C]acetate, [2-^{13}C]acetate, [1,2-^{13}C]acetate, [1-^{13}C]propionate, [1,4-^{13}C]succinate, or [1-^{13}C]butyrate were added to the media after 7 hr of culture and the cultivations were continued at 27° for 48 hr. The broth filtrates (800–1200 ml) were then extracted with chloroform at pH 8.0, and the organic layer was concentrated to dryness. The leucomycin complexes obtained were chromatographed over silica gel thin layer plate using benzene–acetone (2:1) as developer to isolate pure leucomycin A_3 (10–35 mg).

Table I indicates the incorporation ratio of the various ^{13}C-labeled precursors. The ^{13}C NMR spectrum of leucomycin A_3 labeled with [1-^{13}C]acetate showed very strong enrichment for carbons-1, -9, -11, -13, -15, and -20 and much weaker incorporation at carbons-5 and -17. In the experiment with [2-^{13}C]acetate the peaks for carbons-2, -10, -12, -14, -16, and -21 were observed with a strong incorporation while smaller enrichment was evident at carbons-6, -7, -8, -18, and -19. The carbon signal assignments[2] were corroborated by the coupling constants $J_{^{13}C-^{13}C}$ measured in the spectrum of leucomycin A_3 labeled with [1,2-^{13}C]acetate. The spectrum of Ib labeled with [1-^{13}C]propionate revealed only one peak of increased intensity corresponding to C-7. No incorporation of [1,4-^{13}C]succinate into any car-

bon site was observed.

The presence of the weakly enriched peaks resulting from the single labeled acetates was especially interesting. They could be interpreted as the consequence of an indirect incorporation of the precursor through propionate (C-7, C-8, C-19)[3] and butyrate (C-6, C-18 and C-5, C-17). In order to test the hypothesis of the butyrate origin of the indicated four carbons, the simultaneous addition of cold butyrate and [2-^{13}C]acetate was investigated. The resulting ^{13}C NMR spectrum of leucomycin A_3 obtained in this experiment showed an important decrease of the relative intensity of the two signals due to C-6 and C-18 (see Table II) reflecting the partial inhibition of the indirect incorporation of butyrate.

Finally [1-^{13}C]butyrate was added to the culture. The resulting ^{13}C NMR spectrum of Ib indicated strong enrichment, as expected, only for C-5 while weak incorporation was observed under these conditions for all the six carbons originating from the carboxyl carbon of acetate. In analogy with the biosynthetic incorporation of butyrate into the antibiotic X-537 A[4] this result is interpreted as the degradation of butyrate into acetate by β-oxidation.

Our results are clearly in contrast to the conclusions of previous studies[1] in which an acetate origin was assigned to C-3 and C-4. However, the biogenetic origin of these two carbons was not elucidated in the present study since no incorporation was evident at these sites by the precursors used.

Based on the results presented above in Tables I and II we propose the biosynthetic scheme shown in Figure 1 (Ib) for the formation of the aglycone of leucomycin A_3.

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References and Notes

(1) (a) H. Achenbach and H. Grisebach, *Naturforsch.*, **196**, 561 (1964); (b) D. Srinivasan and P. R. Srinivasan, *Biochemistry*, **6**, 3111 (1967); (c) H. Grisebach and C. A. Weber-Schilling, *Naturforsch.*, **23**, 655 (1968); (d) H. Grisebach and W. Hofheinz, *J. R. Inst. Chem.*, 332 (1964).
(2) S. Omura, A. Nakagawa, A. Neszmelyi, S. D. Gero, A.-M. Sepulchre, F. Piriou, and G. Lukacs, *J. Am. Chem. Soc.*, **97**, 4001 (1975).
(3) These carbons did not show increased intensity in the experiment with [1-^{13}C]acetate. Similar differences in behavior of [1-^{13}C]acetate and [2-^{13}C]acetate in the indirect incorporation of propionate-derived carbons were observed in rifamycin biosynthesis: R. J. White, E. Martinelli, G. G. Gallo, G. Lancini, and P. Beynon, *Nature (London)*, **243**, 273 (1973).
(4) J. W. Westley, D. L. Priess, and R. G. Pitcher, *J. Chem. Soc., Chem. Commun.*, 161 (1972).

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Silver Atom–Ethylene Molecular Complex. Matrix Isolation Electron Spin Resonance Study

Sir:

Numerous examples of molecular complexes between olefin molecules and univalent copper or silver cations are known.[1] The formation of a complex between a neutral atom of these elements and an olefin molecule has not been detected, however. We report, in this communication, the electron spin resonance (ESR) spectra of Ag atom–ethylene complex generated within a rare-gas matrix at near liquid helium temperature.

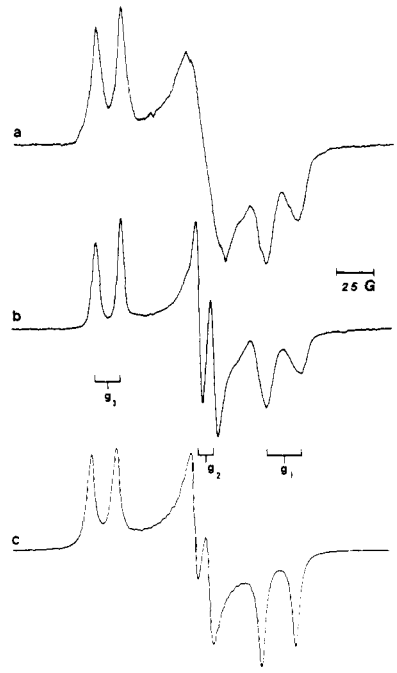

Figure 1. ESR spectra of (a) Ag atom–ethylene (C_2H_4) molecular complexes, (b) Ag atom–ethylene (C_2D_4) molecular complexes, and (c) computer-simulated based upon the parameters given in the text.

The design of the liquid helium cryostat-ESR spectrometer assembly that would permit trapping of high temperature vapor phase species in a rare-gas matrix and observation by ESR of the resulting matrix has been described previously.[2] In the present series of experiments Ag atoms were vaporized from a resistively heated tantalum cell and trapped in a neon matrix together with ethylene molecules introduced through a separate gaseous sample inlet. The composition of the matrix is estimated to be roughly 1000:10:1 for neon atoms, ethylene molecules, and Ag atoms, respectively. The frequency of the spectrometer locked to the sample cavity was 9.410 GHz.

The ESR spectrum of Ag atoms ($4d^{10}5s^1$) isolated in a neon matrix is known.[3] It consists of two sets of sharp, isotropic doublets with the spacings of ~620 and 720 G attributed to ^{107}Ag (natural abundance = 51%, $I = \frac{1}{2}$, $\mu = -0.1130\ \beta_N$), and ^{109}Ag (natural abundance = 49%, $I = \frac{1}{2}$, $\mu = -0.1299\ \beta_N$), respectively. The matrix containing Ag atoms alone appeared white. When trapped together with ethylene, the matrix became red, the ESR signals due to Ag atoms were weak, and a new signal with the overall spread of ~150 G appeared centered about the position corresponding to $g = 2.00$. The new spectrum is assigned to Ag atom–ethylene molecular complexes randomly oriented within the matrix.

The new spectra obtained when normal ethylene (C_2H_4) and perdeuterioethylene (C_2D_4) were used, respectively, are compared in Figures 1a and 1b. The deuteration clearly improves the resolution of the spectrum but does not alter the overall spectral pattern. The latter must hence be attributed to the anisotropic g tensor of the complex and its hyperfine coupling tensor to the Ag nucleus. The following parameters were assessed from the observed spectra.

$$g_1 = 1.972 \pm 0.001$$

$$g_2 = 2.002 \pm 0.001$$

$$g_3 = 2.042 \pm 0.001$$