been described in detail. In brief, a pulsed UV excimer laser beam crosses a broad-band IR beam (Nernst glower) in a static gas cell at 295 K. A monochromator resolves probe frequencies to 5 cm^{-1} and a digitizer captures transient IR absorptions from a 1-MHz InSb detector.

To observe $Cr(CO)_4(C_2H_4)_2$, we first convert $Cr(CO)_6$ into metastable $Cr(CO)_5(C_2H_4)$ by photolysis with excess C_2H_4. FTIR spectra show that $Cr(CO)_6$ (2000 cm^{-1}) decreases with photolysis while new bands at 2085 (~0.03), 1980 (0.82), and 1975 (1.00) cm^{-1} grow in along with lines for free CO. The new bands are assigned to $Cr(CO)_5(C_2H_4)$ and agree well with the few reported $Cr(CO)_5$(olefin) complexes[4] and with more numerous $M(CO)_5$(olefin) (M = Mo, W) complexes.[3]

After $Cr(CO)_6$ is converted to $Cr(CO)_5(C_2H_4)$, laser-induced transient IR absorptions are clearly observed. Figure 1 shows the complete transient absorption spectrum which consists of five bands at 2084, 2045, 1975, 1961, and 1931 cm^{-1}. The negative bands at 2084 and 1975 cm^{-1} are due to parent and match the absorption bands of $Cr(CO)_5(C_2H_4)$. We assign the remaining three positive bands to *cis*-$Cr(CO)_4(C_2H_4)_2$.

We reach this conclusion on the basis of the following observations. The new bands all display a decay constant equal to that for the recovery of $Cr(CO)_5(C_2H_4)$, which indicates a simple interconversion between two species. Coordinatively unsaturated species are excluded on the basis of the long lifetime of the new complex[9] and the inverse dependence of its observed decay constant on ethylene pressure (see below). The three new bands have an integrated intensity ratio of 1.0:2.5:1.4 which excludes the trans isomer. We suspect that the 1931-cm^{-1} band is an unresolved doublet giving a total of four bands.[10] Our observation of *cis*-$Cr(CO)_4(C_2H_4)_2$ is surprising in light of the trans structures for $M(CO)_4(C_2H_4)_2$ (M = Mo, W).[3]

$Cr(CO)_4(C_2H_4)_2$ is expected to relax to $Cr(CO)_5(C_2H_4)$ by a dissociative substitution process:[11]

$$Cr(CO)_4(C_2H_4)_2 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} Cr(CO)_4(C_2H_4) + C_2H_4 \qquad (1)$$

$$Cr(CO)_4(C_2H_4) + CO \xrightarrow{k_3} Cr(CO)_5(C_2H_4) \qquad (2)$$

The assumptions of a steady state for $Cr(CO)_4(C_2H_4)$ and time-independent densities of excess CO and C_2H_4 predict a simple exponential decay for $Cr(CO)_4(C_2H_4)_2$ and recovery for $Cr(CO)_5(C_2H_4)$ with identical time constants. All of the time-dependent absorptions in Figure 1 are well fit by single exponentials and the time constants match under all conditions.

The above kinetics predict the observed decay constants (k_{obsd}) to depend on CO and C_2H_4 pressures:

$$k_{obsd} = \frac{k_1k_3[CO]}{k_2[C_2H_4] + k_3[CO]} \qquad (3)$$

Figure 2 shows that k_{obsd} increases with CO at constant $[C_2H_4]$ becoming nonlinear at high [CO] in accordance with eq 3. Equation 3 also indicates that k_{obsd}^{-1} should be linear with $[C_2H_4]$. This is observed when $[C_2H_4]$ is varied from 300 to 900 torr with CO constant at 0.56 torr. A vanishing intercept is found, but this is expected for $[CO] << [C_2H_4]$. Furthermore, the CO dependence (0.12–1.5 torr) at 700 torr of C_2H_4 is linear, which confirms this supposition.

Under conditions where $k_2[C_2H_4] >> k_3[CO]$, the quantity k_1k_3/k_2 can be determined from the linear dependencies of k_{obsd} on [CO] and $1/k_{obsd}$ on $[C_2H_4]$. The three separate data sets give an average value of $k_1k_3/k_2 = (44.4 \pm 5.1) \times 10^3\ s^{-1}$. From the weighted, nonlinear least-squares fit to the data of Figure 2, separate values of k_1 and k_3/k_2 are found to be $k_1 = (6 \pm 2) \times 10^4\ s^{-1}$ and $k_3/k_2 = 0.7 \pm 0.2$. These combine to give a value of $k_1k_3/k_2 = (46 \pm 18) \times 10^3\ s^{-1}$, in good agreement with the linear fits.

The fast gas-phase unimolecular decay constant obtained for *cis*-$Cr(CO)_4(C_2H_4)_2$ (k_1) establishes the instability of this complex. This behavior differs drastically from that of $Mo(CO)_4(C_2H_4)_2$ and $W(CO)_4(C_2H_4)_2$, which are stable trans isomers.[3] We see no evidence for *trans*-$Cr(CO)_4(C_2H_4)_2$.

The unimolecular decay constant of $Cr(CO)_4(C_2H_4)_2$ is 3×10^7 larger than the reported 300K solution value for $Cr(CO)_4$(η^4-butadiene).[6,12] Complexes of $Cr(CO)_4$ with nonconjugated dienes are more stable than with conjugated dienes,[5a,b,6,13] but our results show that *$Cr(CO)_4$(olefin)$_2$ complexes decay orders of magnitude faster than both.* This result has important implications for the theory of bonding in these complexes.

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Note Added in Proof. After this paper was submitted we became aware of new work by Gregory et al.[14] reporting formation of *cis*-$Cr(CO)_4(C_2H_4)_2$ in xenon solution. The assigned IR spectrum of the cis isomer, as well as crude kinetic observations confirming its formation as the primary photosubstitution product, are in good agreement with the present work. Gregory et al. also report the slower appearance of *trans*-$Cr(CO)_4(C_2H_4)_2$ in their system, which is not observed in our gas-phase experiments (possibly due in our case to pulsed irradiation and the scavenging effect of added CO).

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(9) The gas-phase CO recombination rate constants for metal carbonyl fragments are generally on the order of $10^{10}\ M^{-1}\ s^{-1}$: Seder, T. A.; Church, S. P.; Weitz, E. *J. Am. Chem. Soc.* **1986**, *108*, 4721.

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(12) Reference 6 reports a solution rate constant for the reaction (η^4-butadiene)$Cr(CO)_4 \rightarrow$ (η^2-butadiene)$Cr(CO)_4$, which we compare to our gas-phase value for k_1. However, the data of ref 6 can be interpreted by an alternate mechanism that does not give an elementary rate constant (see ref 5g,h). Efforts are under way in this laboratory to study the gas-phase kinetics of (η^4-butadiene)$Cr(CO)_4$.

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Macrolide Biosynthesis. Tylactone Formation Involves the Processive Addition of Three Carbon Units

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An important question in the biosynthesis of macrolide antibiotics like erythromycin A and tylosin concerns the biochemistry of macrolactone formation.[1] It is clear that simple carboxylic acids—acetate, butyrate, and propionate—are the source of all the carbon and oxygen atoms of the erythronolide[2,3] and tylonolide[4,5] macrolactones and very probable that the α-carboxyl de-

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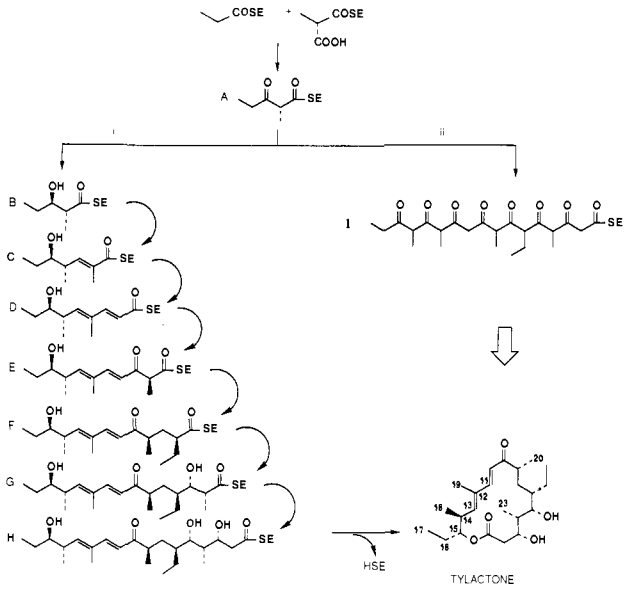
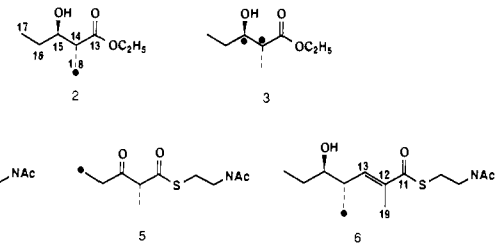


Figure 1. Two hypotheses for the carbon chain assembly mechanism in tylactone biosynthesis. The configurations of the chiral centers of A–H correspond to the absolute stereochemistry of **1**. The "SE" indicates attachment of the intermediates to the enzyme via a thioester bond to the cysteamine portion of coenzyme A, pantetheine, or an acyl carrier protein. In route i or ii the last intermediate in the carbon chain assembly process is converted to **1** by intramolecular lactonization.[5]

rivatives of these acids are key macrolactone assembly intermediates.[2b,4b] But further insight into this complex biochemical process is lacking.

If macrolactone formation resembles the biosynthesis of fatty acids,[6] as currently believed, it would involve assembly of the two to four carbon precursors via enzyme-bound, thioester intermediates. We can imagine two possibilities for such a process in the biosynthesis of tylactone **1** (Figure 1). The first idea (i) shows that the condensation product of propionate and (2*S*)-2-methylmalonate (A) is converted to (2*R*,3*R*)-2-methyl-3-hydroxypentanoate (B), before addition of the next three-carbon substrate. After B condenses with a second 2-methylmalonate, the resulting 2,4-dimethyl-3-oxo-5-hydroxyheptanoate intermediate is reduced and dehydrated to C before its reaction with the next chain-extending, two-carbon unit (malonate). We call this the "processive" mechanism of macrolactone formation because each time carbon chain extension occurs, the resulting β-keto thioester intermediate undergoes no reaction (D → E), reduction only (A → B, F → G → H), reduction and dehydration (B → C → D), or reduction–dehydration–reduction (E → F) before the next chain-extending reaction. It characteristically does not involve the systematic repetition of the same multistep process in extending the carbon chain as in fatty acid biosynthesis. The second idea (ii) shows that A is converted to the poly-β-ketone intermediate I by a series of condensations with malonate, 2-methylmalonate, or 2-ethylmalonate; then I is processed to the macrolactone by an unspecified sequence of reduction and dehydration reactions. Since neither hypothesis could be validated by what is known about the biosynthesis of polyketide metabolites,[7] we examined this question by precursor feeding experiments and now report data which strongly favor the processive mechanism i.

The two concepts for tylactone biosynthesis will be distinguished if any of the compounds drawn in Figure 1, except A, are incorporated into **1** intact. As B was easily accessible synthetically,[8] we devised a synthesis of ethyl (2*R*,3*R*)-2-methyl-3-hydroxypentanoate (supplementary material) that provided optically active material (86–87% ee) with a diastereomeric purity of 88% 2*R*,3*R* and 12% 2*S*,3*R* and ^{13}C labels at the C-2 methyl (**2**) or at C-2 and C-3 (**3**). The results of feeding [2′-^{14}C]-**2** and the sodium



Structures **2**, **3**, **4**, **5**, and **6**. The positions of **1** that correspond to the carbons of these compounds are indicated by the numbering of **2** and **6**. • = ^{13}C label.

salt of its free acid to the *Streptomyces fradiae* GS14 strain[9] revealed that the ethyl ester was more efficiently incorporated into **1** than the acid (8.7× higher total incorporation; 5.3× greater specific incorportation). However, **2** (fed at 7.5 or 60 mM concentrations in the culture medium) did not ^{13}C-enrich C-18 of **1** more than the four other methyl groups (C-17, C-19, C-20, and C-23) which are known to originate from C-3 of propionate.[4] A similar result was obtained when **3** was fed (7.5 mM concentration) to the GS14 strain: C-14 and C-15 of **1** were not ^{13}C-enriched significantly more than any other position derived from C-1 or C-2 of propionate, and the intensity of the $^{1}J_{C_{14}C_{15}}$ doublets obsd. at the C-14 and C-15 resonances in the 125-MHz $^{13}C\{^{1}H\}$ NMR spectrum of the ^{13}C-labeled **1** were too weak to believe that a very small amount of **3** had been incorporated intact into **1**. These results and a similar finding from feeding ethyl [5-^{13}C]-2-methyl-3-oxopentanoate indicated that all three of these precursors had been metabolized to [1-, 2-, or 3-^{13}C]-propionate instead of entering tylactone directly as intact six-carbon units.

Catabolism of precursors larger than acetate or propionate has been an inherent problem with whole cell studies of polyketide metabolism,[10] but a solution was found by using the *N*-acetylcysteamine (NAC) thioester of 2-methyl-3-hydroxypentanoate (**4**). This precursor can more easily undergo transesterification with a thiol group on an enzyme than an oxyester,[11] yet we were not confident that it would survive the fermentation conditions since thioesters are more reactive than oxyesters under several conditions.[12] Nevertheless, the synthesis of **4** from **2** (ca. 40% ee) (supplementary material) and its feeding (13.8 mM concentration) to the GS14 strain resulted in a 6% specific ^{13}C incorporation into **1** with a 1:1.1 ratio of intact-to-catabolic incorporation, based on the increase in the peak heights of the resonances for C-17, C-18, C-19, C-20, and C-23 (Figure 2, supplementary material).

The ability of an NAC thioester to be incorporated intact into tylactone was confirmed by two subsequent findings: that feeding **5** (15 mM concentration) resulted in a 1:2 ratio of intact-to-catabolic incorporation into **1** and that feeding **6** (15 mM concentration; 30% recovery of unabsorbed **6**) resulted in a 0.6% specific ^{13}C incorporation into **1**, based on the increase in the height of the signal for C-18. Since the signals for C-17, C-19, C-21, and

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(10) For example, incorporations intact and after catabolism to acetate have been reported for octanoic acid when it serves as the precursor of coniine, an alkaloid of hemlock plants (Leete, E.; Olson, J. O. *J. Am. Chem. Soc.* **1972**, *94*, 5472–5477), for the hexanoate starter unit of averufin (Townsend, C. A.; Christensen, S. B.; Trautwein, K. *J. Am. Chem. Soc.* **1984**, *106*, 3868–3869), for acetoacetate in nonactin biosynthesis (Clark, C. A.; Robinson, J. A. *J. Chem. Soc., Chem. Commun.* **1985**, 1568–1569), and, recently, for octanoate in fungichromin biosynthesis (Harrison, P. H.; Noguchi, H.; Vederas, J. C. *J. Am. Chem. Soc.* **1986**, *108*, 3833–3834).

(11) It is customary to use thioesters for in vitro studies of fatty acid biochemistry as donors or acceptors of enzyme substrates or products via transesterification; e.g.: Rainwater, D. L.; Kolattukudy, P. E. *J. Biol. Chem.* **1985**, *260*, 616–623.

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C-23 were not enhanced, it appears that **6** was not catabolized to [3-^{13}C]propionate by *S. fradiae*.

Although further experiments using the NAC thioesters of D–G must be done to confirm the implication that tylactone formation is a processive biochemical event, we are encouraged by the above results to make this conclusion. We also predict that the biosynthesis of other macrolide lactones, e.g., 6-deoxyerythronolide B, will be proven to occur in an analogous manner.[13] Consequently, the results indicate that the biochemistry of macrolactone formation can be studied systematically with the expectation of learning how the enzymology controls the sequence of precursor assembly and creates the absolute stereochemistry of these structurally complex natural products.

Acknowledgment. This research was supported by a grant from Eli Lilly and Co. We thank Professor D. Seebach for a gift of ethyl (−)-3-hydroxypentanoate; Dr. Bruce Adams, Department of Chemistry, University of Wisconsin—Madison, for the 125-MHz ^{13}C NMR spectra; Dr. Narayan Punekar for feeding **5** and **6**; and Drs. E. T. Seno and R. H. Baltz for helpful discussions, bacterial strains, fermentation media constituents, and a sample of tylactone.

Supplementary Material Available: Experimental data for the synthesis of compounds **2–6**, the precursor feedings, Figure 2, and the method of calculating the intact-to-catabolic incorporation ratios for **4** and **5** (10 pages). Ordering information is given on any current masthead page.

(13) See the following paper by Cane and Yang *J. Am. Chem. Soc.*, following paper in this issue.

Macrolide Biosynthesis. 4. Intact Incorporation of a Chain-Elongation Intermediate into Erythromycin

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Although the general outlines of erythromycin biosynthesis are by now well accepted, the details of the key chain-elongation process by which the macrolide carbon skeleton is assembled are still obscure.[1] Extensive investigations in our own[2] and other laboratories[1] have demonstrated the role of propionate as the basic building block for the macrolide aglycone and established that the oxygen atoms at C-1, C-3, C-5, C-9, C-11, and C-13 are all derived from the propionate precursor. These results have been interpreted in terms of a mechanism analogous to the chain-elongation steps of fatty acid biosynthesis in which the oxidation level and stereochemistry of the growing polyketide chain are adjusted subsequent to each condensation step involving successive units of methylmalonate (Scheme I). Further support for the analogy to fatty acid biosynthesis has come from the finding that incorporation of [2-^{2}H$_2$,2-^{13}C]propionate gave erythromycin A (**1**) and B (**2**), which retained deuterium at C-2, C-4, and C-10, consistent with the direct utilization of (2*S*)-[2-^{2}H,2-^{13}C]-methylmalonyl-CoA (**3b**) in those condensation steps leading to the generation of D-methyl groups,[3] as required by a process involving decarboxylative inversion.[4] Unfortunately, all of these experiments, limited to simple three- and four-carbon precursors, have provided only indirect insights into the details of the chain-elongation process itself. Indeed, none of the intermediates between methylmalonate and the parent aglycone, 6-deoxyerythronolide B (**8**), have ever been directly detected, either in antibiotic-producing cultures of *Streptomyces erythreus* or in mutants blocked in the formation of **8**, nor do any of the latter mutants act as secretors in cosynthesis experiments.[5] Furthermore, although the structural genes for erythromycin biosynthesis have recently been cloned,[6] a viable cell-free synthetase mediating formation of the macrolide skeleton has so far eluded numerous attempts at isolation. We now report the first successful incorporation of a partially elaborated polyketide into the intact macrolide ring of erythromycin.

According to the proposed mechanism for erythromycin biosynthesis (Scheme I), chain elongation is initiated by condensation of (2*R*)-methylmalonyl-CoA (**3a**) (or an enzymatically equivalent thioester) with propionyl-CoA to give (2*S*)-2-methyl-3-ketopentanoyl-CoA (**4**). Stereospecific reduction of **4** will then give (2*S*,3*R*)-2-methyl-3-hydroxypentanoyl-CoA (**5**), which serves as the substrate for the next condensation reaction. We sought, therefore, to test this mechanism by attempted incorporation of (2*S*,3*R*)-[2,3-^{13}C$_2$]-2-methyl-3-hydroxypentanoic acid (**9**), readily available by the versatile methodology for enantiospecific aldol condensations recently introduced by Evans.[7,8] The use of the double ^{13}C label not only provides greater sensitivity in the detection of low levels of enrichment, it also allows recognition of incorporation of the intact precursor against the anticipated background due to substrate degradation and random incorporation of derived propionate. Thus [2′-^{13}C]-*N*-propionyloxazolidone (**10**), prepared as described from [2-^{13}C]propionyl chloride[9] and (4*S*)-4-(2-propyl)oxazolidone,[7] was treated with di-*n*-butylboryl triflate and diisopropylethylamine, and the derived *Z* boron enolate was condensed with anhydrous [1-^{13}C]propionaldehyde[10] in CH_2Cl_2 followed by oxidative workup (Scheme II). The resulting (2′*S*,3′*R*)-[2′,3′-^{13}C$_2$]-*N*-(2-methyl-3-hydroxypentanoyl)oxazolidone (**11**), obtained in 65% yield, appeared as a single diastereomer by ^{1}H and ^{13}C NMR analysis, displaying a characteristic $J_{H2,H3}$(erythro) = 2.9 Hz.[7,12,13] Hydrolysis of

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(7) (a) Evans, D. A.; Bartoli, J.; Shih, T. L. *J. Am. Chem. Soc.* **1981**, *103*, 2127. (b) Evans, D. A.; Nelson, J. V.; Vogel, E.; Taber, T. R. *J. Am. Chem. Soc.* **1981**, *103*, 3099. (c) Evans, D. A. *Aldrichimica Acta* **1982**, *15*(2), 23.

(8) The preparation of **9** has been reported by Masamune: Masamune, S.; Choy, W.; Kerdesky, F. A. J.; Imperiali, B. *J. Am. Chem. Soc.* **1981**, *103*, 1566.

(9) [2-^{13}C]Propionyl chloride was prepared by treatment of sodium [2-^{13}C]propionate (99 atom % ^{13}C, MSD Isotopes) with 1.4 equiv of phthaloyl chloride (150 °C, 1.5 h) followed by heating to 200 °C and collection of the distillate.

(10) Reduction of [1-^{13}C]propionic acid (99 atom % ^{13}C, Cambridge Isotopes) with 1.2 equiv of borane–dimethyl sulfide (Et_2O, reflux, 6 h) gave the [1-^{13}C]tripropyloxyboroxine[11] trimer which was oxidized with 1.1 equiv of PDC in a minimum volume of CH_2Cl_2 (50 °C, 2 h). Distillation of the reaction mixture and passage of the distillate through Florosil gave [1-^{13}C]-propionaldehyde (70 mg/mL, 40% yield). Success in the subsequent aldol condensation reaction required that the propionaldehyde solution be no more dilute than 10% as well as free of water and contaminating propanol and propionic acid.

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(12) The configurational assignments were based on the known stereochemical course of the boron enolate mediated aldol condensation of **10**, derived from L-valine,[7] as well as the characteristic ^{1}H and ^{13}C NMR parameters: (J(erythro) = 3–6 Hz, J(threo) = 7–9 Hz; δ C-2 methyl (erythro) 9–13, δ C-2 methyl (threo) 12–18). Cf.: Evans, D. A.; Nelson, J. V.; Taber, T. R. In *Topics in Stereochemistry*; Allinger, N. L., Eliel, E. L., Wilen, S. H., Eds.; Wiley: New York, 1982; pp 1–115. Heathcock, C. H. In *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic: New York, 1984; Vol. III, pp 111–212.

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