Revision of the Biosynthetic Origin of Oxygens in Mevinolin (Lovastatin), a Hypocholesterolemic Drug from *Aspergillus terreus* MF 4845

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Mevinolin (**1**) (also known as lovastatin, monacolin K, and Mevacor) is a widely prescribed drug for reduction of plasma cholesterol levels in humans and is produced by fermentation of fungi such as *Aspergillus terreus*.[1] The biosynthetic arrangement of its bonds derived from the carbons, oxygens, and hydrogens of acetate and methionine has been examined previously by our group through incorporation of precursors multiply labeled with stable isotopes (Figure 1).[2] Those results support a polyketide pathway[3] for its construction, which may include an intriguing enzyme-catalyzed Diels–Alder reaction to form the bicyclic system.[2b,4] More recent studies by Endo and co-workers have elucidated the late postassembly transformations (oxidations and side chain attachment) leading from 4a,5-dihydromonacolin L (**2**) to **1** in *Monascus ruber*.[5] Apparently no bicyclic precursor less oxidized than **2** has yet been reported. An unusual feature of our earlier biosynthetic work was the failure to detect intact incorporation of the carbon–oxygen bonds derived from sodium [1-^{13}C,$^{18}O_2$]acetate into the expected positions C-11, C-13, and C-15 on the main chain of mevinolin (**1**) using ^{18}O-induced isotope shifts in ^{13}C-NMR spectra.[2b] The only ^{18}O label clearly observed in that experiment was at the C-1′ carbonyl oxygen. In accord with this result, fermentation of *A. terreus* ATCC 20542 in an atmosphere of $^{18}O_2$ gas showed incorporation of up to four ^{18}O atoms into **1** by mass spectral analysis (Table 1), thereby indicating that the oxygens at C-8, C-11, C-13, and C-15 were aerobically derived. Unfortunately, this result could not be confirmed by NMR isotope shift techniques because biological production of **1** by this fungal strain had declined to very low levels. Recently careful subculture selection from a new strain (*A. terreus* MF 4845) and improved fermentation media have increased yields of **1** to about 200 mg per liter of culture. Since detailed knowledge of the biosynthetic origins of atoms and bonds in polyketides is a prerequisite for design and intact incorporation of advanced intermediates (e.g., di-, tri-, and tetraketides),[3b,4b,6] we have reinvestigated the incorporation of sodium [1-^{13}C,$^{18}O_2$]acetate and $^{18}O_2$ gas into mevinolin (**1**) by *A. terreus* MF 4845. The results of the present study indicate that the oxygens at C-11, C-13, and C-15 are in fact acetate derived, at least in this organism, and that **2** may be the final product released from the polyketide synthase complex.

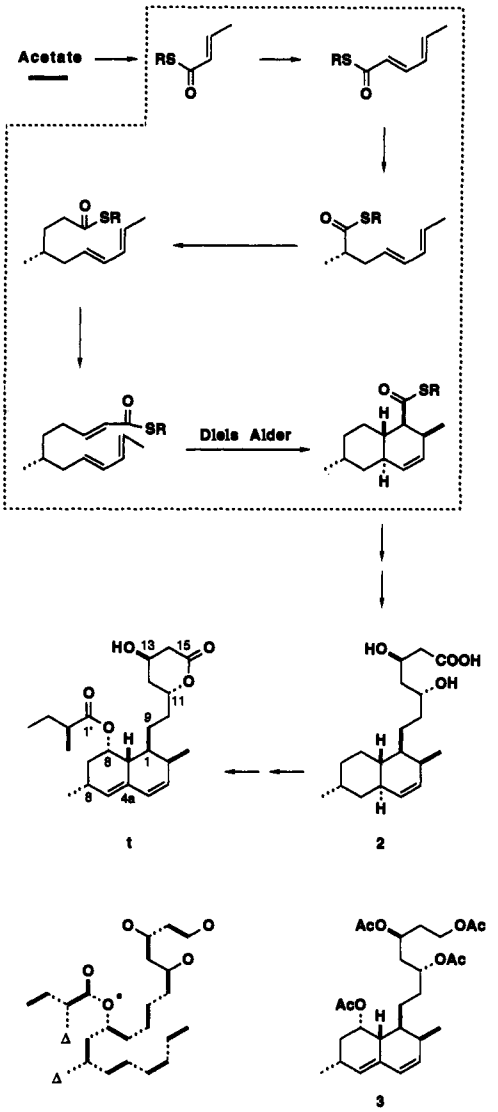

Figure 1. Proposed biosynthetic pathway to mevinolin (**1**) via **2**. Potential di-, tri-, tetra-, penta-, and hexaketide intermediates in the assembly process are indicated as enzyme-bound thiol esters (boxed structures) at the oxidation stage attained immediately before addition of the next two-carbon unit. The hepta- and octaketide stages are not shown, but the nonaketide thiol ester may resemble **2**. Carbons (Δ) from the methyl of methionine and from oxygen (•) of $^{18}O_2$ (current experiments) are indicated, as is the arrangement of acetate units (—).

Fermentation of *A. terreus* MF 4845 in an atmosphere containing $^{18}O_2$ gas as described previously[2b] produced a sample of **1** whose chemical ionization mass spectrum shows incorporation of one ^{18}O atom per molecule (32% incorporation based on M + 2 peak), in contrast to the previous results. Isotope shifts in the ^{1}H-decoupled ^{13}C-NMR spectrum (75 MHz) of this material also indicate that only the oxygen attached to C-8 (at δ 68.0; Δδ 0.034) is detectably labeled. In a separate experiment, sodium [1-^{13}C,$^{18}O_2$]acetate (isotopic purity 90% ^{13}C, 90–97% ^{18}O) was incorporated into cultures of the same organism in a normal atmosphere. Examination by ^{13}C-NMR as before showed a measurable isotope shift for the doubly-bonded oxygen at C-1′ (at δ 176.9; Δδ 0.038), but displayed only broadened signals for C-11, C-13, and C-15. Since two-bond carbon–carbon couplings

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Table 1. Mass Spectral Data for **1** Derived from $^{18}O_2$ Fermentations of *A. terreus*

m/z (**1** from ATCC 20542)[a]	intensity, % obs[c]	(calc)[d]	m/z (**1** from MF 4845)[b]	intensity, % obs[c]	(calc)[d]
415 (MH + 10)[a]	1	(0)	432 (MNH_4 + 10)[b]	0	(0)
414	2	(0)	431	0	(0)
413 (MH + 8)	8	(0)	430 (MNH_4 + 8)	0	(0)
412	2	(0)	429	0	(0)
411 (MH + 6)	9	(0)	428 (MNH_4 + 6)	0	(0)
410	3	(0)	427	2	(0)
409 (MH + 4)	12	(0.06)	426 (MNH_4 + 4)	11	(0.06)
408	16	(0.57)	425	14	(0.58)
407 (MH + 2)	64	(4.54)	424 (MNH_4 + 2)	51	(4.64)
406	27	(27.1)	423	28	(27.5)
405 (MH)	100	(100)	422 (MNH_4)	100	(100)

[a] Chemical ionization spectra acquired using isobutane; see ref 2b for details. [b] Chemical ionization spectra acquired using ammonia; other procedures and conditions as in ref 2b. [c] Observed ion intensities (±1.5%) for **1** derived for $^{18}O_2$. [d] Calculated nominal ion intensities (%) for unlabeled **1**. Mass spectral values for unlabeled **1** are in agreement within experimental error.

due to multiple incorporation of labeled acetates into the same molecule of **1** could cause such broadenings, spin-echo experiments were used to separate coupled and uncoupled resonances.[7] Although this further clarified the isotope shift at C-1′ and suggested that C-11 and C-13 might be labeled, the latter two resonances remained broad and ill-defined, as did C-15. To elucidate the possible labeling pattern, **1** was reduced with lithium aluminum hydride to a tetraol, which was then converted to the corresponding tetraacetate **3**. The magnitudes of ^{18}O isotope shifts are dependent on structure, with alcohols and ethers having relatively small shifts which can be increased by attachment of an electron-withdrawing group (e.g., acetyl).[8] The ^{13}C-NMR spectrum of **3** displayed clear isotope shifts for C-11 (at δ 70.9; $\Delta\delta$ 0.037), C-13 (at δ 68.2; $\Delta\delta$ 0.036), and C-15 (at δ 60.5; $\Delta\delta$ 0.030) (Figure 2), thereby demonstrating that the bonds to oxygen at these carbons originate from acetate. It appears that the peak broadening in the ^{13}C-NMR spectra of **1** may be primarily due to hydrogen-bonding or exchange processes which mask the small isotope shifts. Considerable loss of ^{18}O occurs relative to the amount of ^{13}C (ca. 1% enrichment per site; 2-fold signal enhancement) incorporated during the biosynthetic process, presumably by exchange processes. In an attempt to suppress this, the sodium [1-^{13}C,$^{18}O_2$]acetate was converted to its *N*-acetylcysteamine thiol ester because it had been shown previously such that derivatization aids loading of advanced intermediates into polyketide synthases.[3b,6] However, the incorporation was only slightly higher than with the corresponding sodium acetate.

The present results demonstrate that the carbon–oxygen bonds at C-11, C-13, and C-15 of **1** can be derived intact from acetate and indicate that the primary pathway to this polyketide proceeds via reduction at these sites only to the alcohol stage prior to addition of the next C_2 unit. Hence, our original suggestion[2b] of late stage aerobic oxidations at these positions is incorrect, and **2** may be the first compound released after assembly of the

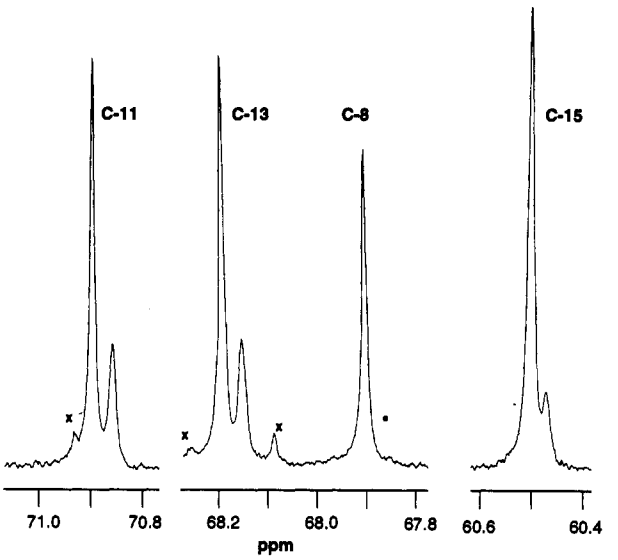

Figure 2. Expansions of ^1H-decoupled ^{13}C NMR spectra (100 MHz) of tetraacetate **3** showing C-11, C-13, C-8, and C-15. ^{13}C-Coupled signals are indicated by "x" where visible. See the supplementary material for experimental details.

skeleton. The failure to detect the acetate oxygen incorporation previously was apparently due to the difficulty of observing the very small isotope shifts in **1**. The high level of oxygen exchange at these sites relative to carbon label incorporation suggests that acetate (or products in biochemical equilibrium with it) may undergo metabolism which could account for the unprecedented incorporation of aerobic oxygen with *A. terreus* ATCC 20542 reported earlier.[9] The results provide a cautionary note for incorporation experiments with ^{18}O. Current experiments with advanced precursors to verify the occurrence of a biological Diels–Alder reaction during biosynthesis of **1** will be reported later.

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Supplementary Material Available: Description of experimental details (5 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(9) In the previous $^{18}O_2$ experiments (ref 2b) only small quantities of **1** were produced. The possibility of compartmentalized generation of [^{18}O]-acetate by oxidative degradation of a larger acetate-derived precursor was considered and discounted because β-oxidation normally adds oxygen from water in the medium. Although it could not be fully verified with the present system, we believe that formation of ^{18}O-labeled acetate or malonate must have occurred because mass spectral analysis of **1** labeled by $^{18}O_2$ in the original experiments was repeated several times by various methods. Examination of the new results (with strain MF 4845) in Table 1 also shows an unexpectedly high intensity (11%) for the (MNH_4 + 4) peak, suggesting the presence of some molecules having two ^{18}O atoms. Apparently an uncharacterized shunt pathway leading to [^{18}O]acetate must account for the additional aerobic labeling.