dinuclear complexes of Rh(I) is currently being explored in our laboratory and will be reported in detail in future publications.

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Supplementary Material Available: Tables of kinetic and equilibrium data and thermodynamic and activation parameters for reaction 4 and a listing of atomic positional and thermal parameters for $(PEt_3)_2(CO)Rh-Co(CO)_4$ (3 pages). Ordering information is available on any current masthead page.

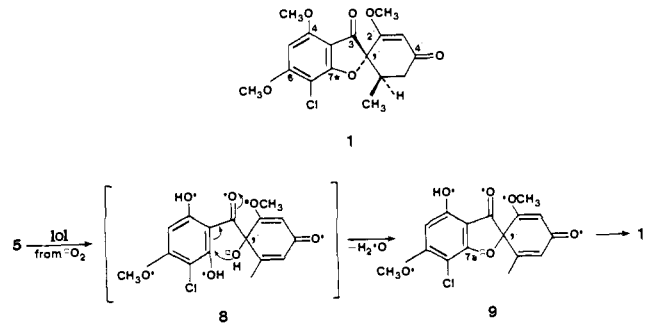
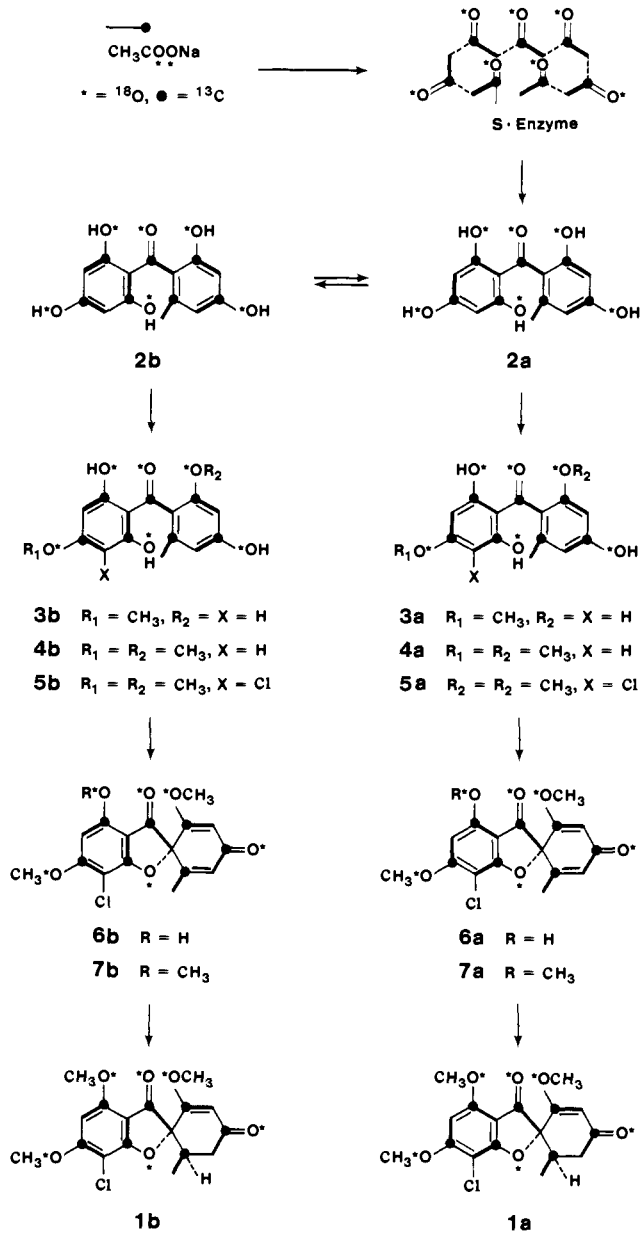


Figure 1.

Scheme I



Biosynthetic Source of Oxygens in Griseofulvin. Spin-Echo Resolution of ^{18}O Isotope Shifts in ^{13}C NMR Spectroscopy†

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Griseofulvin (**1**) is a commercially important antifungal antibiotic from *Penicillium* species[1] which has antimitotic[2] and porphyrogenic[3] activity. These biological effects and its unusual spiro structure have inspired a number of elegant syntheses[4] and biosynthetic studies.[5] However, despite the emergence of a coherent biogenetic picture displayed in Scheme I which is supported by incorporation of advanced precursors into griseofulvin (**1**),[5d,e] the possibility persisted that some of the proposed intermediates represent shunt metabolites[6] because of lack of knowledge about intermediate oxidation states. Although the development of ^{13}C NMR spectroscopy has greatly advanced our understanding of Nature's assembly of complex carbon skeletons from simple precursors,[7] the exact sequence and mechanism of oxidative and reductive transformations have frequently remained obscure, primarily due to inability to easily detect functional group changes during biosynthesis. Fortunately, partial conquest of this problem now seems imminent with the arrival of two new methodologies: ^2H NMR spectroscopy[5b,8] and detection of ^{18}O labels by upfield

† Dedicated to Professor George H. Büchi, Massachusetts Institute of Technology.

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isotope shifts induced in ^{13}C NMR spectra.[9-11] The latter technique has already seen use in nonbiological mechanistic work[10] as well as in biosynthetic studies on averufin,[11a] cytochalasin B,[11b] citrinin,[11c] lasalocid A,[11d] monensin A,[11e] and erythromycins A and B.[11f] Ironically, a common difficulty is excessive incorporation of the ^{13}C,^{18}O precursor which leads to multiple ^{13}C labels within a single molecule. The resulting long-range carbon–carbon couplings often obscure the small ^{18}O-induced isotope shifts. In some cases this has been avoided by diluting the ^{13}C,^{18}O precursors with unlabeled carriers.[11e,f] In the present study we describe a solution to this problem using a spin-echo sequence[12] in ^{13}C NMR spectroscopy to identify the biosynthetic source of all of the oxygens in griseofulvin (**1**).

Earlier work has shown that the carbon skeleton of griseofulvin (**1**) is synthesized from seven intact acetate units in two arrangements, **1a** and **1b**,[5c,13] and that the last step is probably stereospecific reduction of cooccurring dehydrogriseofulvin (**7a–b**).[14] Although the ease of direct chemical oxidative coupling[4c] of the hydroxylated benzophenone, griseophenone A, to **7** strongly supports the biogenetic path **5** → **6** → **7** proposed by Harris,[5d] an alternative possibility (Figure 1) would involve aerobic oxidation of **5** to hydroxy quinoid intermediate **8** which could cyclize to **9** by an addition–elimination process.[15] The two routes differ in the origin (acetate or atmosphere) of the oxygen bridging C-7a and C-1′ in **1**. Presence of acetate oxygens at the other sites would exclude participation of reduced (deoxygenated) intermediates which undergo aromatic hydroxylation in later stages of biosynthesis. To distinguish between these possibilities, we incorporated[16] doubly labeled sodium [1-^{13}C,$^{18}O_2$]acetate[17] (isotopic purity:[18] 90% ^{13}C, 18% $^{18}O_1$, 81% $^{18}O_2$) into griseofulvin (**1**) with growing cultures of *Penicillium griseofulvum ATCC 11885*.

Expansion of the 100.6-MHz ^{13}C NMR spectrum offered dismaying portraits of the ^{13}C-labeled carbons; the potential isotope shifts were cloaked by a complex system of couplings resulting from incorporation of multiple labels within the same molecule (Figure 2). For example, for C-7a of **1** both the ^{13}C–^{16}O and ^{13}C–^{18}O signals would appear as singlets if no ^{13}C were present two bonds away, but parts of each of these signals couple to C-4, C-6, and C-3 because of multiple ^{13}C substitution. Fortunately spin-echo Fourier transform[12] (SEFT) ^{13}C NMR spectroscopy allowed separation of ^{12}C–C–^{13}C–(^{16}O or ^{18}O) and ^{13}C–C–^{13}C–(^{16}O or ^{18}O) pairs. The pulse sequence for the experiment is $(90°-\tau-180°-\tau-\text{acquisition-T})_N$ where τ is set to $1/(2J_{CC})$, T is the delay between acquisition and the next pulse sequence, and N is the number of accumulations. In this case J_{CC} is the approximate two-bond coupling constant, which was estimated from the normal ^{13}C NMR spectrum assuming a reasonable value for the ^{18}O isotope shift.[9c-g,19] Using this sequence the ^{13}C–C–^{13}C–(^{16}O or ^{18}O) resonances (doublets) are phase modulated as a function of J_{CC} and are inverted (relative to the normal ^{13}C NMR spectrum) at $\tau = 0$, 90° out of phase at $\tau = 1/(4J_{CC})$, and in phase (positive) at $\tau = 1/(2J_{CC})$. The ^{12}C–C–^{13}C–(^{16}O or ^{18}O) resonances (singlets) always remain inverted regardless of the τ value used.[20]

Application of the SEFT technique resolved all of the signals by inverting the uncoupled singlets (Figure 2) and showed that every oxygen in griseofulvin (**1**) originates from acetate with preservation of the carbon–oxygen bonds (Table I). Comparison of the intensities of the ^{13}C–^{16}O and ^{13}C–^{18}O singlets in the SEFT spectra with ^{13}C–^{16}O signals in the spectra of unlabeled **1** suggests that a maximum of two-thirds of the ^{18}O label at each site may be lost by exchange with fermentation medium.[21] Such quantitative estimates must be viewed with extreme caution since the exact influences of SEFT on relative signal intensities are still uncertain. In addition, appearance of several labeled acetate units in the same griseofulvin molecule is not entirely random because the pulse-feeding technique[16] which was used momentarily swamps the acetate pool with carbon-13. During this time the chance of multiple incorporation of ^{13}C (and ^{18}O) is high. This leads to an excess of coupled signals (which are not inverted in the SEFT spectrum) for the [^{18}O]griseofulvin relative to the ^{16}O species. The isotope ratios in Table I therefore represent minimum incorporation of ^{18}O and probably suggest more exchange than has actually occurred. We are currently investigating these aspects in more detail.

Our results exclude involvement of the hydroxy quinoid intermediate **8** as well as of partly deoxygenated intermediates which would have to be hydroxylated with atmospheric oxygen in later biosynthetic steps. Presence of acetate oxygen at all sites in griseofulvin (**1**) supports previously suggested[5] Scheme I which portrays Claisen-aldol condensations of a heptaketide followed by tautomerization to form **2**. Stepwise methylation of the two rotamers **2a** and **2b** leads (via **3** → **4** → **5**) to oxidative closure (**5** → **6**), another methylation (**6** → **7**), and eventual reduction

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(17) This compound was prepared from sodium [1-^{13}C]acetate (KOR Isotopes) and [^{18}O]H_2O (Stable Isotopes Resource, Los Alamos National Laboratory) by repeated conversion to [1-^{13}C,$^{18}O_2$]acetic acid[10f] and neutralization with sodium [^{18}O]hydroxide in [^{18}O]H_2O. A more convenient synthesis has been developed by Cane and coworkers.[11e]

(18) Extent of labeling was determined by conversion to the corresponding *p*-bromophenacyl ester (Moses, C. G.; Reid, E. E. *J. Am. Chem. Soc.* **1932**, *54*, 2101–2103) followed by mass spectrometric and ^{13}C NMR analysis. The latter procedure showed four peaks (intensities 1:9:9:81) for the ^{13}C-labeled carbon corresponding to (^{16}O—^{13}C=^{16}O), (^{18}O—^{13}C=^{16}O), (^{16}O—^{13}C=^{18}O), and (^{18}O—^{13}C=^{18}O) species. The upfield isotope shifts relative to the first peak were 0.0110, 0.0375, and 0.0485 ppm, respectively.

(19) The magnitude of the coupling constants (J_{CC}) was confirmed by examination of ^{13}C NMR spectra of [^{18}O]griseofulvin derived from sodium [1-^{13}C]acetate.

(20) A full description of this technique will be published elsewhere.

(21) Spectra of [^{13}C,^{18}O]griseofulvin (**1**) were normalized to spectra of unlabeled material and corrected for natural abundance contribution of ^{13}C, ^{16}O, and isotopic purity of the precursor.

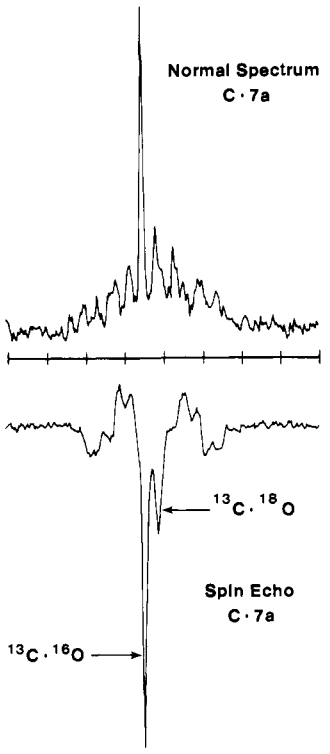

Figure 2. 100.6-MHz ^{13}C NMR spectral expansions of C-7a of griseofulvin (**1**) derived from sodium [1-^{13}C,$^{18}O_2$]acetate (0.05 ppm/division). The spin-echo experiment (5684 scans) has $\tau = 0.109$ s.

Table I. ^{13}C NMR Data of Griseofulvin (1) Derived from Sodium [1-^{13}C,$^{18}O_2$]Acetate[a]

carbon	chemical shift, ppm	$\Delta\delta^{16}O-^{18}O$ (ppm × 100)[b]	isotope ratio[c] $^{16}O/^{18}O$
4'	196.8	4.2	68:32
3	192.4	4.0	75:25
2'	170.8	2.0	83:17
6	169.7	1.5	78:22
7a	164.7	1.7	75:25
4	157.9	1.6	71:29

[a] Fourier transform spectra were measured on a Bruker WH400 spectrometer at 100.6 MHz by using ca. 0.1 M solutions of **1** in $CDCl_3$ with Me_4Si internal standard. Assignments agree with those reported in ref 5c. Signals for carbons bearing oxygens were first expanded by using 32K data block/2000 Hz, 80–1200 scans, 16.4-s acquisition time, and 45° pulse angle. Subsequent spin-echo expansions (vide infra) employed 32K data block/2000 Hz, 650–5700 scans, and 8.2-s acquisition time. [b] ^{18}O upfield shift values are ±0.1 (ppm × 100). [c] Approximate values obtained from relative peak heights of $^{13}C-^{16}O$ and $^{13}C-^{18}O$ inverted signals in the spin-echo experiments.

to **1**. The use of spin-echo Fourier transform (SEFT) techniques for separation of ^{18}O-induced isotope shifts from long-range carbon–carbon couplings has already proved advantageous in other biosynthetic work in our laboratories and should be widely applicable.

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A Fluxional η^1-Cycloheptatrienyl Derivative of Ruthenium Exhibiting Both 1,2 and 1,4 Metal Migrations

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The fluxional behavior of a large number of compounds in which a cyclopentadienyl ring is bonded in monohapto fashion to a metal has been studied.[1,2] In all cases where the pathway has been determined, the metal migrates to the adjacent carbon atom of the five-membered ring; that is, a 1,2 migration occurs. In no case has an alternate pathway been detected, although the possibility of two or more concurrent processes was recognized at an early stage.[3]

We recently prepared the first η^1-cycloheptatrienyl derivative of a transition metal, $(OC)_5Re(7\text{-}\eta^1\text{-}C_7H_7)$ (**1**),[4] and showed that it was fluxional by 1,2 migration of the metal. In further investigations, we have now prepared the ruthenium derivative $(\eta^5\text{-}C_5H_5)Ru(CO)_2(7\text{-}\eta^1\text{-}C_7H_7)$ (**2**).[5] Remarkably, this monohapto compound displays two concurrent fluxional pathways, 1,2 *and* 1,4 migration.

Synthesis of **2** followed the same anion plus tropylium route (reaction 1) used previously.[4] Infrared spectroscopy indicated

$$Na[(\eta^5\text{-}C_5H_5)Ru(CO)_2] + [C_7H_7][BF_4] \xrightarrow[-78\,^\circ C]{THF} (\eta^5\text{-}C_5H_5)Ru(CO)_2(\eta^3\text{-}C_7H_7) \quad (1)$$

2

nearly quantitative formation of **2**, but isolated yields were 70% or less due to the very high solubility in pentane from which it was crystallized at −78 °C.[6,7]

The fluxionality of **2** was studied by applying the spin saturation transfer technique of Forsén and Hoffman[9] to the ^{13}C NMR spectrum[10] at 279.0 K.[11] Spectra acquired with saturation at

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(5) Compound **2** is an orange crystalline solid, mp 47 °C. Anal. Calcd for $C_{14}H_{12}RuO_2$: C, 53.67; H, 3.86. Found: C, 53.63; H, 3.86. Mass spectrum (14 eV, 25 °C), M^+, $(M-CO)^+$, $(M-2CO)^+$, $C_7H_7^+$ (base peak); IR (cyclohexane, ν_{CO}, cm^{-1}) 2015 (s), 1961 (s), 1958 (sh); ^1H NMR (cyclohexane-d_{12}, 20 °C) δ 5.40 (s, C_5H_5), 5.64 (m, $H_{3,4}$), 5.22 (m, $H_{1,6}$), 5.10 (m, $H_{2,5}$), 4.02 (t, H_7, $^3J_{1-7} = 7.8$ Hz); olefinic proton resonances (cf. Figure 1 for numbering system) assigned by decoupling experiments. ^{13}C NMR (THF-d_8, 20 °C) δ 202.7 (CO), 140.8 ($C_{1,6}$), 134.9 ($C_{3,4}$), 122.8 ($C_{2,5}$), 89.9 (C_5H_5), 19.5 (C_7); assignments derived from ^1H NMR by selective decoupling.

(6) Compound **2** in solution slowly forms ditropyl and the ruthenium dimer; it is slightly less stable thermally than **1**. The ruthenium anion was obtained by a published procedure.[8]

(7) The result for ruthenium may be contrasted with the analogous reaction involving iron in which mainly $[(\eta^5\text{-}C_5H_5)Fe(CO)_2]_2$ was obtained with a very low yield (4%) of a compound ultimately formulated as $(\eta^5\text{-}C_5H_5)Fe(CO)(\eta^3\text{-}C_7H_7)$: Ciappenelli, D.; Rosenblum, M. *J. Am. Chem. Soc.* **1969**, *91*, 3673, 6876. In our hands, this reaction gives the iron dimer quantitatively.

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