and potassium attachment ions resulting from adventitious salts also appear in this spectrum.

These results suggest that FTMS measurement conditions may permit mass spectral measurements of some molecules found to be highly labile when analyzed by TOF mass spectrometry. Differences of the present data from previous observations of the metastable behavior of ions derived from Chl *a* may be due to (a) excess energy imparted in the fission fragment desorption process, (b) intervention of relatively slow chemical ionization reactions with rates incompatible with TOF analysis, or (c) selectivity of FTMS analysis, which may favor trapping of only low-energy ions. Although the 1-V trapping potentials employed ensure that ions within the cell with 1 eV or less translational energy components along the magnetic field axis are trapped, they need not have low internal energy. However, ions that have undergone many collisions will tend to have both lower internal energy and lower translational energy and may therefore be favored in FTMS. Thus, LD-FTMS should be complementary to methods using TOF mass spectrometry.

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Nonstereospecific Hydrogen Exchange in the Biosynthesis of the Macrolide Antibiotic, Brefeldin A

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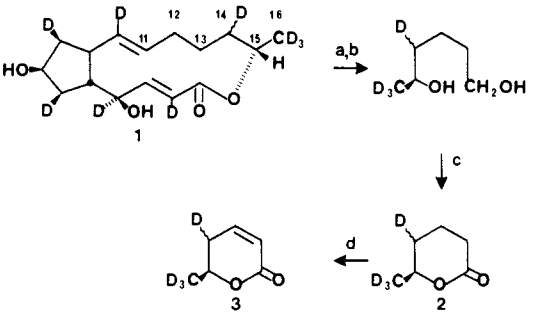
The results of isotopic labeling experiments show that the carbon and hydrogen atoms of simple carboxylic acid precursors like acetate label corresponding sites in fatty acids and macrolides but partial loss of isotopic hydrogen from the α-position of these precursors has been noted in several cases.[1] For saturated fatty acids this was found not to be due to simple exchange from malonyl-CoA in vitro,[2] which led to the belief that the loss of hydrogen results from a "post-malonate" exchange process.[2a] It was suggested that this loss may require the involvement of a base in the enzyme but possibly one that does not have a role in fatty acid biosynthesis.[2a] If the latter were true, then the exchange should be stereospecific but difficult to observe, because one of the two hydrogens of the prochiral malonyl thio ester intermediate is removed during the subsequent steps of the pathway. We now show, in contrast, that this exchange is nonstereospecific for the biosynthesis of brefeldin A (**1**), a fungal macrolide that is made from acetate and malonate in a manner similar to the fatty acids.[3]

Brefeldin A, which had been labeled by [$^2H_3$]acetate in vivo,[4] was degraded (Scheme I) to the (5*S*)-2,4,6-trideuterio lactones **2** and **3**. Since C-4 of **2** or **3** corresponds to C-14 of **1**, it was possible to show by ^2H NMR spectral analysis at 30.7 and 76.8 MHz that the C-4 prochiral positions of **2** (Figure 1c) and **3** (spectral data not shown)[5] contained approximately equal amounts of ^2H.[6a] Mass spectral analysis of [^2H]-**2** established that C-4

Scheme I[a]



[a] (a) O_3/MeOH, −78 °C; (b) $LiAlH_4$/THF; 90% combined yield; (c) Pt/O_2, $NaHCO_3$; 54% yield; (d) LDA/THF, −78 °C; PhSSPh, $NaIO_4$, 110 °C; 45% yield.

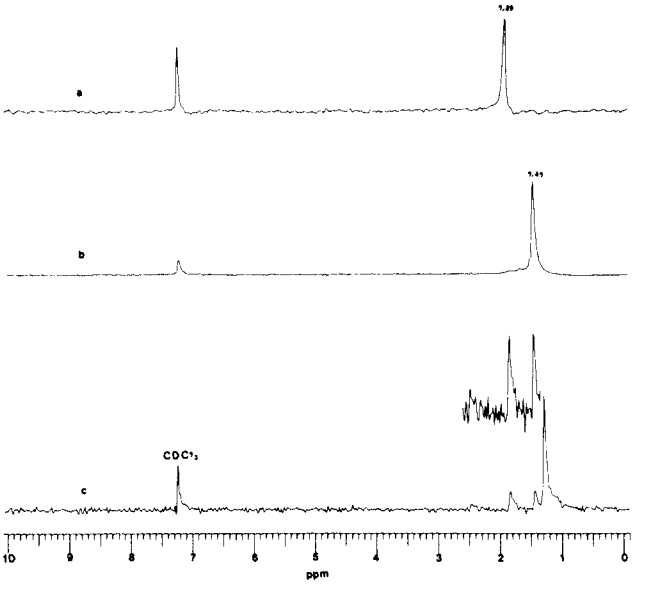


Figure 1. ^2H NMR spectra at 76.8 MHz in $CDCl_3/CHCl_3$ of (a) [^2H]-**5**, (b) -**6**, and (c) -**2**. The signal at 1.89 in (a) is for the ^2H at C-4, at 1.45 in (b) for the ^2H at C-4, and at 1.28 ppm in (c) for the [^2H]methyl group attached to C-5.

was labeled predominantly with one ^2H atom.[6b] Therefore, the spectral data shown in Figure 1c resulted mostly from an approximately equal mixture of molecules labeled intermolecularly at the 4-*pro-R* or 4-*pro-S* positions, rather than from molecules labeled intramolecularly at both of these positions.

Samples of [4,6-$^2H_5$]-**4**, (4*R**)-[4-^2H]-**5**, and (4*S**)-[4-^2H]-5-methylvalerolactone (**6**) were synthesized (Scheme II) to provide reference compounds for the spectral assignments used in making the above deductions. Their 2,3-unsaturated derivatives were made by the method used to prpare **3** from **2** (Scheme I). Analysis of the proton NMR spectrum of 5-methylvalerolactone and its 2,3-unsaturated derivative by double-resonance experiments, followed by comparison of these data with the ^1H, ^2H, and ^{13}C NMR spectral data for **4**–**6** resulted in the spectral assignments given in Table I. This information therefore identified the chemical shifts of ^2H attached to the 4-*pro-R*-, 4-*pro-S*-, and 5-positions of **4**–**6**.

Brefeldin A, consequently, must have been equally labeled at its 14-*pro-R*- and 14-*pro-S*-positions by the [$^2H_3$]acetate, which means that ^2H was lost nonstereospecifically from the malonate (derived from the acetate) that provided carbons 13 and 14 of **1**. This result is the opposite of our earlier speculation[3] and implies that the loss of hydrogen during macrolide biosynthesis occurs

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(5) The corresponding ^2H NMR spectrum of **3** showed a signal at 1.32 and two equally intense signals at 2.18 and 2.30 ppm.

(6) (a) We could distinguish between mixtures of **5** and **6** in 50:50 and 43:57 ratios by integration of the appropriate signals in the 30.7-MHz ^2H NMR spectra. (b) The isotopic composition of the *m/z* 99 ($M^+ - 15$) fragment ion in the high-resolution mass spectrum of **2** was 97.5% d_0, 2.0% d_1, and 0.52% d_2.

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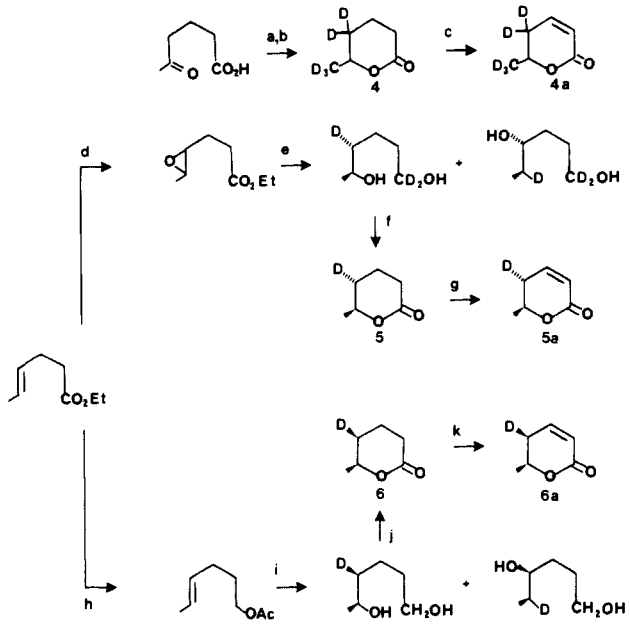
Table I. ^{1}H NMR Spectral Data for Compounds 2-6[a]

compd	H-2	H-3	H-4 *pro-R*	H-4 *pro-S*	H-5	C-6 CH_3
2	2.5 (m)[b]	1.8-2.0 (m)	1.2-2.0 (m)	1.5 (m)	4.43 (m)	1.36 (d, 6.5)
3	5.94 (ddd, 1.19, 2.53, 9.82)	6.82 (ddd, 5.34, 2.68, 9.82)	2.28 (m)	2.28 (m)	4.51 (dqd, 4.46, 6.55, 10.72)	1.37 (d, 6.55)
4	2.5 (m)	1.8-2.0 (m)	c	c	4.5 (m)	c
5	2.5 (m)	1.8-2.0 (m)	c	1.51 (m)	4.42 (m)	1.36 (d, 6.28)
5a	5.98 (dd, 2.68, 9.82)	6.84 (dd, 2.38, 9.82)	c	2.25 (m, 2.38, 2.68, 11.76)	4.53 (m, 6.55, 11.76)	1.40 (d, 6.55)
6	2.5 (m)	1.8-2.0 (m)	1.89 (m)	c	4.4 (m)	1.35 (d, 6.16)
6a	6.0 (dd, 0.9, 9.53)	6.85 (dd, 5.66, 9.82)	2.33 (m, 0.9, 3.87, 5.66)	c	4.56 (m, 3.87, 6.55)	1.42 (d, 6.26)

[a] Spectra were determined at 90, 200, or 270 MHz in $CDCl_3$; assignments are given in parts per million relative to the $CHCl_3$ at δ 7.24 as the internal standard. [b] Signal multiplicities and assignable coupling constants (Hz) are given in parentheses. [c] No signal because of ^{2}H substitution.

Scheme II[a]



[a] (a) NaOD, D_2O/dioxane; (b) $NaBH_4$/dioxane; H^+; 77% combined yield; (c) LDA/THF, -78 °C; PhSSPh, $NaIO_4$, 110 °C; 69% yield; (d) mCPBA/CH_2Cl_2, 0 °C; 52% yield; (e) $LiAlH_4/Et_2O$, 0 °C; 52% yield of 1,5-diol and 98% combined yield; (f) Pt/O_2, $NaHCO_3$; H^+; 28% yield from epoxide; (g) same as (c); 60% yield; (h) $LiAlH_4/Et_2O$; Ac_2O, DMAP (catalyst), CH_2Cl_2; 80% yield; (i) THF:BD_3; H_2O_2/NaOH; 38% yield of 1,5-diol and 80% combined yield; (j) same as (f); 18% yield from olefin acetate; (k) same as (c); 51% yield.

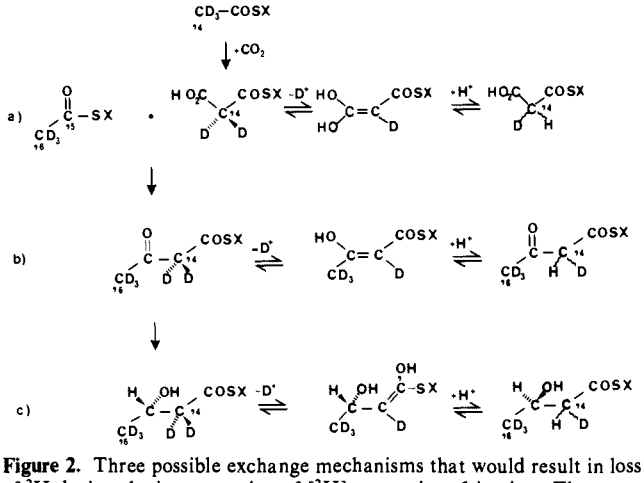


Figure 2. Three possible exchange mechanisms that would result in loss of ^{2}H during the incorporation of [^{2}H]acetate into **1** in vivo. The numbering of intermediates in (a), (b), and (c) corresponds to the positions of **1** that would be labeled by the acetate; X = coenzyme A or enzyme.

primarily at the level of the malonate intermediates and probably nonenzymatically. Malonyl thio esters would be likely candidates since their α-hydrogens are known to be exchangeable easily at physiological pH's.[2a,7,8a] Thus among the three possibilities shown in Figure 2 for proton (deuteron) exchange involving thio ester intermediates, we favor (a). Further hydrogen exchange might occur during the reduction of enoyl thio ester intermediates by analogy to observations made for fatty acid biosynthesis.[8] For brefeldin A, however, this possibility must have a very minor importance since all the positions labeled by [$^2H_3$]acetate (excluding the C-16 methyl group) exhibited a similar amount of ^{2}H loss, including the two, C-6 and C-8, where reduction of enoyl thio esters was postulated to occur.[9]

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Novel Hypervalent (10-I-2) Iodine Structures

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Studies of hypervalent iodine species have focused chiefly on iodinane (10-I-3), periodonium (10-I-4), and periodinane (12-I-5) systems.[1,2] With the exception of the trihalide anions, examples of stable 10-I-2 compounds are rare. The metal-halogen exchange reaction, discovered independently by Gilman[3] and Wittig,[4] has been the subject of numerous studies and mechanistic speculation,

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