sulfonamides containing primary alkyl substituents. As shown in the table, the β-alkoxy-substituted lithium reagent **2h** can be successfully prepared and trapped, without undergoing loss of Li alkoxide, to give fluoroalkene **3h** under these reaction conditions. We are continuing to explore the scope of this procedure with regard to the preparation of other fluorine-substituted enol ethers.

Acknowledgment. We acknowledge support for this work given by the National Institutes of Health.

Registry No. 1a, 42599-17-7; **1b**, 42599-24-6; **1c**, 81793-05-7; **1d**, 101166-41-0; **1e**, 101166-42-1; **1f**, 101166-43-2; **1g**, 101166-44-3; **1h**, 83114-95-8; **1i**, 101198-43-0; **3a**, 32814-17-8; **3b**, 20405-77-0; **3c**, 101166-45-4; **3d**, 101166-46-5; **3e**, 101166-47-6; **3f**, 101198-44-1; **3g**, 101166-48-7; **3h**, 101166-49-8; **3i**, 101198-45-2; $C_6H_5SO_2N(F)C(CH_3)_3$, 101166-50-1.

Laser Desorption Fourier Transform Mass Spectrometry of Chlorophyll *a* and Chlorophyll *b*

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Received December 27, 1985

The mass spectral fragmentation behavior of chlorophyll *a* (Chl *a*) was the subject of several recent reports which concluded that intact molecular ions are metastable, with lifetimes on the microsecond time scale.[1-3] Chait and Field,[1,2] using ^{252}Cf fission fragment ionization coupled with a time-of-flight (TOF) mass spectrometer, concluded >99% of molecular ions undergo unimolecular decomposition in less than 67 μs. Tabet et al. reached similar conclusions in their TOF analysis of CO_2 laser desorption of Chl *a*.[3] These studies have serious implications regarding the applicability of laser-desorption Fourier transform mass spectrometry (LD-FTMS), with its inherently longer measurement time scale (>10 ms), to the study of large labile moelcules. Thus, the present study was undertaken to determine whether it would prove possible to observe such species by LD-FTMS.

Positive ion LD mass spectra of Chl *a* and chlorophyll *b* (Chl *b*)[4] were obtained by using a Nicolet FTMS 1000 mass spectrometer which was described previously.[5] Samples were prepared by evaporation of methanol solutions containing approximately 100 μg of Chl *a* or *b* onto a 12.7-mm diameter circular stainless steel direct insertion probe tip. For doped samples, an equal amount of KBr was added to the solution. Each spectrum was obtained from a single CO_2 laser pulse which was focused onto an area of ca. 1 mm^2 on the probe tip. Ions thus produced were trapped by using 1-V trapping potentials for 1.5–2.0 s while desorbed neutrals were pumped away. For improved mass resolution in the spectra presented, a low mass cutoff of 450 amu (100 KHz) was employed. Other measurements revealed few fragment ions at lower masses.

Figure 1 is the high mass region of the mass spectrum of KBr-doped Chl *a*. All four major ions are potassium attachment ions, with the intact molecular ion species (*m/z* 931) clearly predominant. Other ions at *m/z* 653 $[M + K - (phytyl - H)]^+$, *m/z* 621 $[M + K - phytyl - OCH_3]^+$, and *m/z* 577 $[M + K - phytyl - OCH_3 - CO_2]^+$ correspond to simple fragmentations similar to those previously observed. However, these results

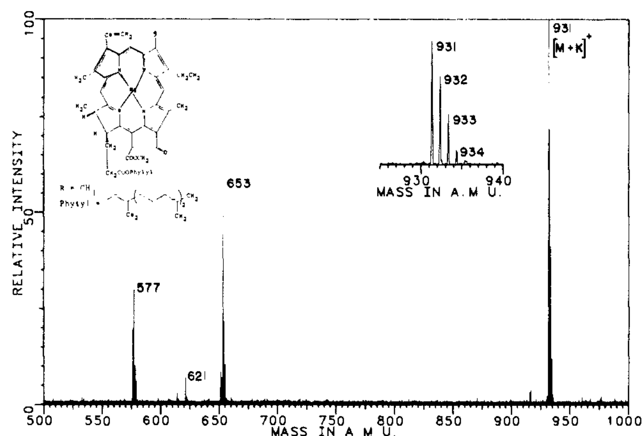

Figure 1. Laser desorption FTMS spectrum of chlorophyll *a* doped with KBr.

Table I. Mass Spectrum of Chlorophyll *b* (doped with KBr)

nominal mass	rel ion intensity	proposed ion struct
945.6	99.3	$[M + K]^+$
667.2	52.4	$[(M + K - (phytyl - H)]^+$
663.6	47.4	*a*
635.6	33.1	$[M + K - phytyl - OCH_3]^+$
628.3	20.6	$[M - (phytyl-H)]^+$
625.5	25.0	*a*
609.5	100.0	$[M + K - CHCOOphytyl]^+$
593.5	13.3	*a*
590.2	14.9	*a*
569.3	8.4	$[M - CH_2COOphytyl]^+$

a Not assigned.

Table II. Mass Spectrum of Chlorophyll *b* (no dopant)

nominal mass	rel ion intensity	proposed ion struct
945.5	5.0	$[M + K]^+$
929.5	3.5	$[M + Na]^+$
907.5	11.7	$[M + H]^+$
906.5	9.0	$[M]^+$
651.2	5.8	$[M - (phytyl - H) + Na]^+$
628.2	100.0	$[M - (phytyl - H)]^+$
597.2	16.8	$[M - (phytyl - H) - OCH_3]^+$
596.2	11.6	$[M - phytyl - OCH_3]^+$
575.2	7.9	$[M - (phytyl - H) - OCH_3 - Mg + H_2]^+$
574.2	5.9	$[M - phytyl - OCH_3 - Mg + H_2]^+$
569.2	33.4	$[M - CH_2COOphytyl]^+$
553.2	8.7	$[M - (phytyl-H) - OCH_3 - CO_2]^+$
552.2	8.8	$[M - phytyl - OCH_3 - CO_2]^+$
541.2	7.4	$[M - CH_2COOphytyl - CO]^+$
495.1	18.7	$[M - CHO - COOphytyl - COOCH_3]^+$
481.2	21.9	$[M - CHO - CH_2COOphytyl - COOCH_3]^+$
467.1	8.4	$[M - CHO - CH_2CH_2COOphytyl - COOCH_3]^+$

contrast with the spectra obtained in the earlier LD-TOF study which showed similar dominant alkali attachment ions (Na^+, K^+, and Li^+) but significantly more fragmentation.[3] These differences probably arise primarily from the much different experimental time scale of the LD-TOF and LD-FTMS measurements, as well as the higher laser power densities in the LD-TOF work.

A plausible explanation for the long-term stability of potassium-attached molecular ions in the LD-FTMS experiment regime is their possible formation by relatively slow, low-energy chemical ionization processes during and following the desorption of neutral molecules. Tables I and II summarize the positive ion spectra of Chl *b* with and without KBr dopant. Of particular significance is the observation that fragmentation is much more evident in the spectrum of undoped Chl *b*. However, the presence of molecular ion $[M]^+$ in 9% relative abundance and $[M + H]^+$ at about 6% relative abundance (after correcting for ^{13}C contributions) demonstrates that alkali attachment is not necessary for observation of Chl *b* molecular ions under LD-FTMS conditions. Sodium

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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.

Acknowledgment. This work was supported by NIH Grant GM-30604, which is gratefully acknowledged.

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

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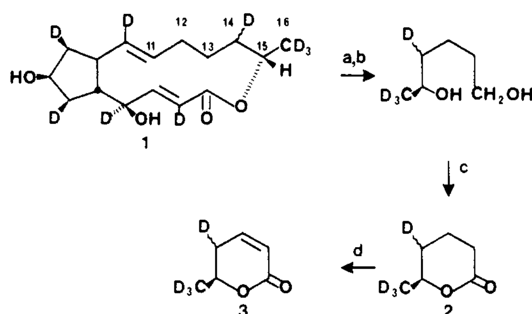
Received November 18, 1985

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 ^{2}H 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 ^{2}H.[6a] Mass spectral analysis of [^{2}H]-**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.

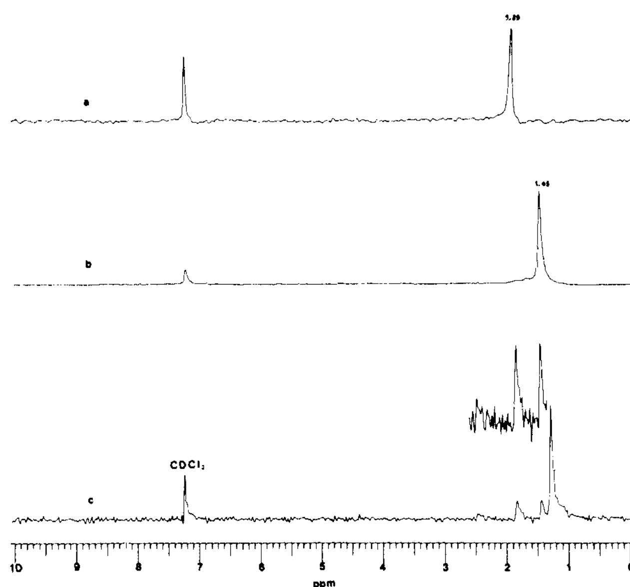


Figure 1. ^{2}H NMR spectra at 76.8 MHz in $CDCl_3/CHCl_3$ of (a) [^{2}H]-**5**, (b) -**6**, and (c) -**2**. The signal at 1.89 in (a) is for the ^{2}H at C-4, at 1.45 in (b) for the ^{2}H at C-4, and at 1.28 ppm in (c) for the [^{2}H]methyl group attached to C-5.

was labeled predominantly with one ^{2}H 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-^{2}H]-**5**, and (4*S**)-[4-^{2}H]-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 ^{1}H, ^{2}H, 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 ^{2}H 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 ^{2}H 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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(2) (a) Sedgwick, B.; Cornforth, J. W. *Eur. J. Biochem.* **1977**, *75*, 465. (b) Sedgwick, B.; Cornforth, J. W.; French, J.; Gray, R. T.; Kelstrup, E.; Willadsen, P. *Ibid.* **1977**, *75*, 481. (c) Arnstadt, K. I.; Schindlbeck, G.; Lynen, F. *Eur. J. Biochem.* **1975**, *55*, 561.
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(4) For general fermentation and feeding procedures, see: Mabuni, C. T.; Garlaschelli, L.; Ellison, R. A.; Hutchinson, C. R. *J. Am. Chem. Soc.* **1979**, *101*, 707.
(5) The corresponding ^{2}H 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 ^{2}H 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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