aldehyde dimethyl acetal, isopropyl alcohol, and methyl acetate, all of which were identified from their GLPC retention times.[11] Those components could also be recognized in the ^{1}H NMR spectrum of the mixture. The carbonyl compounds and isopropyl alcohol were also identified from their gas-phase IR spectra.[12] These results confirm that **1a** decomposes via a carbonyl ylide intermediate in methanol.

Thermolysis of **1a** at 82 °C in 1,1-diphenylethylene (**4**) gave acetone (**5**), methylacetate (**6**), 2,2-dimethyl-1,1-diphenylcyclopropane (**7**)[13] [^{1}H NMR (CCl_4/Me_4Si) δ 1.12 (s, 2 H), 1.25 (s, 6 H), 7.20 (m, 10 H)], and a compound tentatively identified as 1-methoxy-1-methyl-2,2-diphenylcyclopropane (**8**) [^{1}H NMR (CCl_4/Me_4Si) δ 1.16 (m, 4 H), 1.25 (d, ^2J = 9 Hz, 1 H), 3.10 (s, 3 H), 7.30 (m, 10 H); MS, m/z 238 (M^+)]. Those products are readily explained in terms of dimethyl- and methylmethoxycarbene intermediates. Dimethylcarbene was also suggested by the finding of propene from thermolysis of **1a** in benzene.[14] The decomposition of **1a** in CCl_4, which gives products **5**, **6**, and **9**–**11** (Scheme I),[15] provided further support for carbenic JA5M89Ci

Although the only direct evidence for formation of a carbonyl ylide pertains to methanol solvent, it is very likely that the same intermediate is formed in CCl_4 and C_6H_6. The similar magnitudes of the first-order rate constants (above) would have to be fortuitous if different mechanisms were in operation.[17] Moreover, it is now fairly clear[19] that the very similar **1b** forms a carbonyl ylide intermediate in CCl_4 and the intermediate undergoes an intramolecular H transfer as proposed by Shimizu and Bartlett[10a] for analogous processes involving carbonyl ylide intermediates, which can be trapped. Thus **1a** is unlikely to be mechanistically different in the rate-determining step. The sharp difference between **1a** and **1b**, insofar as the product-determining steps are concerned, can be accounted for nicely in terms of the expected donor substituent effect of the OCH_3 group, analogous to that of the NH_2 group.[2]

In order for the ylide intermediate to fragment thermally to carbenes and carbonyl compounds, it must either have a nonplanar ground state[2] or a nonplanar state must be readily accessible from a planar ground state. Calculations[2] indicate that a donor substituent reduces the barriers to rotation of 0°,0° conformations to 0°,90° conformations. It is interesting that there is apparently little preference for one fragmentation over the other (CCl_4 results). The theory[2] for amino-substituted carbonyl ylide predicts that the 0°,90° conformation would have the shorter bond between amino-substituted carbon and carbonyl oxygen. This feature is presumably offset in the presence case by the greater stabilization that the donor substituent affords to a carbene as compared to a carbonyl compound.

Acknowledgment. We are grateful to the Natural Sciences and Engineering Research Council for financial support of this research and to a referee for alerting us to a reference.

(11) These products were separated by GLPC, and their structures were assigned by injecting aliquots to which pure standards had been added, one at a time.

(12) Nicolet, Model 7199, FT-IR instrument fitted with Varian Aerograph 3700 gas chromatograph. Authentic samples were injected under the same conditions to obtain reference spectra.

(13) The ^{1}H NMR spectrum of **7** is in good agreement with that of a model compound, 1-(hydroxymethyl)-1-methyl-2,2-diphenylcyclopropane: Dreibelbis, R. L.; Khatri, H. N.; Walborsky, H. M. *J. Org. Chem.* **1975**, *40*, 2074.

(14) Identified from its ^{1}H NMR spectrum and also by bromination to 1,2-dibromopropane.

(15) Yields and characterization of **9**–**11**. **9**: 31%; ^{1}H NMR (δ, CCl_4/Me_4Si) 5.2 (d, $^2J_{HH}$ = 4 Hz, 1 H), 4.4 (d, $^2J_{HH}$ = 4 Hz, 1 H), 4.0 (s, 3 H); MS, m/z 178, 176, 174 in ratio 0.3:1.0:1.0 (M^+), 121, 119, 117 in ratio 0.3:1.0:1.0 (CCl_3^+), 57 ($C_3H_5O^+$). **10**: 33%; ^{1}H NMR (δ, $CDCl_3$/Me_4Si) 2.0 (s); MS, m/z 163, 161, 159 in ratio 0.3:1.0:1.0 [$(CH_3)_2CCCl_3^+$], 79, 77 in ratio 0.3:1.0 [$(CH_3)_2CCl^+$], mp 180 °C (lit.[16] mp 178.6–179.6 °C). **11**: 30%.

(16) Rogers, A. O.; Nelson, R. E. *J. Am. Chem. Soc.* **1936**, *58*, 1028.

(17) A small decrease of the rate constant for thermolysis of a cis azo compound as solvent polarity is increased is now well-known and explicable in terms of the large dipole moment of the substrate.[18]

(18) Schulz, A.; Rüchardt, C. *Tetrahedron Lett.* **1976**, 3893.

(19) Before the paper of Shimuzu and Bartlett[10a] appeared, one of us[20] had proposed a diradical intermediate to account for the thermolysis products of **1b**.

(20) Yeung, D. W. K.; MacAlpine, G. A.; Warkentin, J. *J. Am. Chem. Soc.* **1978**, *100*, 1962.

Biosynthesis of Macrolide Antibiotics. 3.[1] Regiochemistry of Isotopic Hydrogen Labeling of Brefeldin A by Acetate[†]

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Although the biosynthesis of 14- and 16-membered macrolide antibiotics by microorganisms has been studied for many years, most of the knowledge gained has been about the biochemically simple C_2–C_4 carbon precursors.[2] Scant information is available about the biochemical transformations subsequent to the chain-forming condensation of the C_2–C_5 thioesters leading to the aglycons[3] of these macrolides. This is in distinct contrast to fatty acid biosynthesis in living systems for which a comprehensive mechanistic and enzymological picture can be drawn[4,5] and from which bioorganic mechanisms of macrolide antibiotic formation[2d] have been formulated by analogy.

As the structural intricacies of macrolide antibiotics[2b,6] imply complex biosynthetic routes, it is of fundamental importance to map their anabolic pathways. The 16-carbon macrolide antibiotic brefeldin A (**1**) has been chosen for study, because its biochemical precursors are acetate and malonate.[7] Thus comparisons are possible with the biochemistry of fatty acids which also share these precursors. We summarize in this and the following paper the regio- and stereochemical results obtained about its biosynthesis by isotopically labeling **1** with [2-^{13}C,2-$^2H_3$]-, [1-^{14}C,2-^{3}H]-, and [2-$^2H_3$]acetate.

In our initial experiments we wished to determine whether enrichment from [^{2}H]- and [^{3}H]acetate produced a labeling pattern for **1** similar to that observed for fatty acids.[8] Radioactively labeled **1**, obtained from the metabolism of sodium [1-^{14}C,2-^{3}H]acetate (^{3}H/^{14}C ratio = 9.18) by *Penicillium brefel-*

[†]NRCC No. 19055.

(1) Part 2: Mabuni, C. T.; Garlaschelli, L.; Ellison, R. A.; Hutchinson, C. R. *J. Am. Chem. Soc.* **1979**, *101*, 707–714.

(2) Reviews: (a) Vanek, Z.; Majer, J. In "Antibiotics"; Gottlieb, D., Shaw, P. D., Eds.; Springer Verlag: New York, 1967; Vol. 2, pp 154–188. (b) Omura, S.; Nakagawa, A. *J. Antibiot.* **1975**, *28*, 401–433. (c) Martin, J.-F. *Annu. Rev. Microbiol.* **1977**, *31*, 13–38. (d) Masamune, S.; Bates, G. S.; Corcoran, J. W. *Angew. Chem., Int. Ed. Engl.* **1977**, *16*, 585–607. (e) Corcoran, J. W. *Annu.* Rep. *Med. Chem.* **1977**, *12*, 130–139.

(3) Most of our knowledge is about post-aglycon biochemical processing such as oxidation, glycosidation, and methylation: cf. ref 2 d,e.

(4) Reviews: (a) Volpe, J. J.; Vagelos, P. R. *Annu. Rev. Biochem.* **1973**, *42*, 21–60. (b) Numa, S.; Yamashita, Ş. *Curr. Top. Cell. Regul.* **1974**, *5*, 198–246. (c) Bloch, K.; Vance, D. *Annu. Rev. Biochem.* **1977**, *46*, 263–298.

(5) (a) Sedgwick, B.; Cornforth, J. W. *Eur. J. Biochem.* **1977**, *75*, 465–479. (b) Sedgwick, B.; Cornforth, J. W.; French, S. J.; Gray, R. T.; Kelstrup, E.; Willadsen, P. *Ibid.* **1977**, *75*, 481–495. (c) Sedgwick, B.; Morris, C.; French, S. J. *J. Chem. Soc., Chem. Commun.* **1978**, 193–194. (d) Sedgwick, B.; Morris, C. *Ibid.* **1980**, 96–97.

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(8) (a) Rous, S. *Adv. Lipid Res.* **1971**, *9*, 73–118. (b) Reference 5a and citations therein. (c) White, R. H. *Biochemistry* **1980**, *19*, 9–15. (d) McInnes, A. G.; Walter, J. A.; Wright, J. L. C. *Tetrahedron Lett.* **1979**, 3245–3248.

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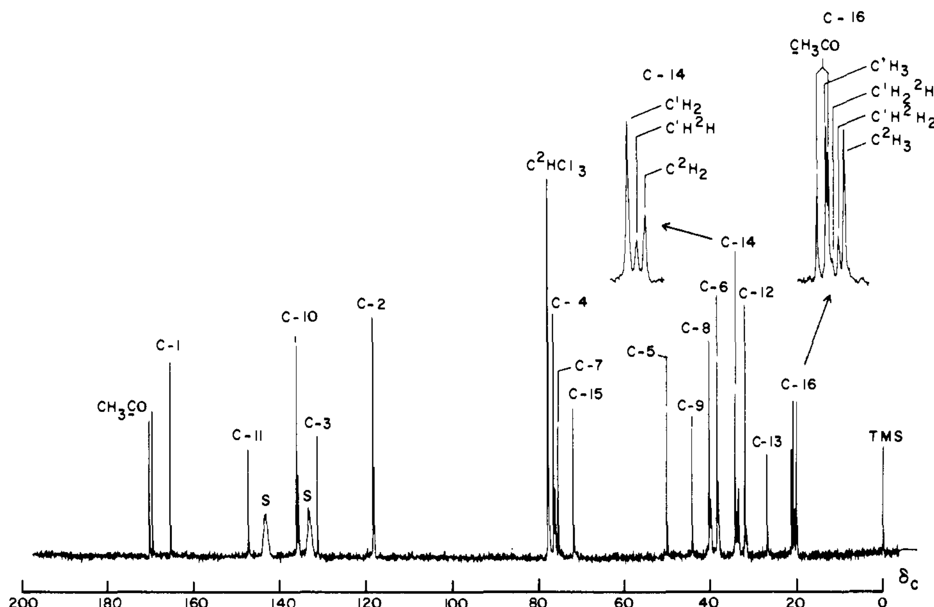


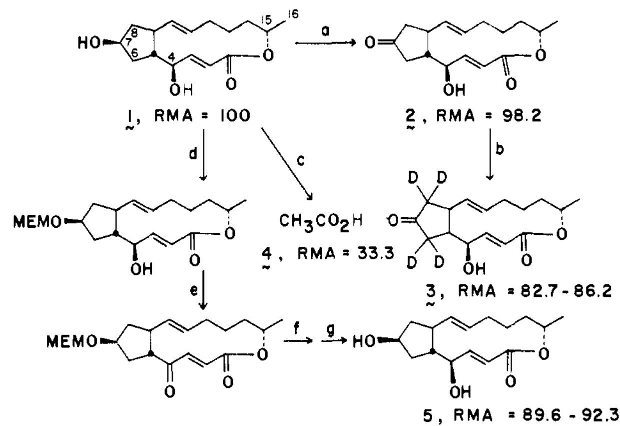
Figure 1. ^{1}H,^{2}H-broadband-decoupled ^{13}C NMR spectrum at 25.2 MHz of **6** (141 mg) in C^2HCl_3-C_6F_6 (4:1), 0.5 mL, containing 5 mg of Cr(acac)$_3$; temperature 30 °C, spectral width 5120 Hz, acquisition time 1.6 s, flip angle 40°, 90° pulse length 44 μs, delay between data acquisitions 8 s during which the ^{1}H decoupling field was switched off for suppression of residual nuclear Overhauser enhancement (NOE), digital resolution ±0.3 Hz, 4700 acquisitions; $\gamma H_2/2\pi$ (^{1}H decoupling, 100 MHz) ca. 3500 Hz, with 0–180° phase modulation at 150 Hz; $\gamma H_2/2\pi$ (^{2}H, 15.4 MHz) ca. 310 Hz, phase modulation 40 Hz; ^{19}F internal lock to C_6F_6. A spectrum was also obtained with ^{1}H decoupling only to confirm the location of ^{2}H isotopically shifted resonances. Assignments of resonances in the ^{13}C spectrum were based on chemical shift trends in broadband ^{1}H decoupled spectra, ^{13}C–^{1}H spin-spin couplings observed in high-resolution spectra, off resonance ^{1}H decoupling experiments, and ^{13}C–^{13}C spin-spin coupling information obtained from spectra of brefeldin A enriched from [1,2-^{13}C]acetate.

dianum Dodge (NRRL 2083) for 3 days,[1] had a ^{3}H/^{14}C ratio of 2.58. This value indicates that the metabolic incorporation of acetate (absolute incorporation of 1.5% based on ^{14}C) had been accompanied by a 71.9% loss of ^{3}H, which agrees well with the observations that [^{3}H]acetate loses 62.5–67.7% of its ^{3}H content on incorporation into palmitate and stearate by the purified fatty acid synthetases from chicken liver and yeast in vitro.[5a] If all isotope effects accompanying the incorporation of [^{14}C,^{3}H]acetate into **1** are ignored, the theoretical ^{3}H/^{14}C ratio for **1** labeled by [^{14}C,^{3}H]acetate should be 3.83 as a result of the loss of two tritium atoms for each of the seven acetate equivalents incorporated into the C-1–C-14 portion of **1**. Thus, the loss of ^{3}H upon the in vivo incorporation of [^{3}H]acetate into **1** was 32.6% more than predicted.

Samples of the [^{14}C,^{3}H]-**1** were degraded chemically[9] to compounds **2–5** (Scheme I) to determine the relative molar radioactivity (RMA) present as ^{3}H at carbons 4, 6–8, and 16. If it is assumed that there is three times more ^{3}H at C-16 than at the other even-numbered carbons, the theoretical RMA values at the five carbons of **1** are C-4, 10%; C-6, 10%, C-7, 0%; C-8, 10%; C-16, 30%. The RMA values for **2** indicate that an insignificant amount of ^{3}H resided at C-7, which should acquire hydrogen only from NADPH,[4a,5d] and that less than 2% of the exchangeable ^{3}H at C-6 and C-8 was lost during the oxidation of **1** to **2**. Subsequent base-catalyzed replacement of ^{3}H by ^{2}H in **2** (done twice) gave **3** containing four deuterons (MS, ^{1}H NMR) and established that C-6 and C-8 of **2** contained 2 equiv of ^{3}H within acceptable experimental error. The RMA of the acetate **4** derived from C-15,C-16 of **1** by Kuhn–Roth oxidation suggests that this C_2 unit was the starting point for assembly of the polyketide chain.[7c,10] Finally, the RMA of **5** shows that 1 equiv of ^{3}H was at C-4.

The results of our partial chemical degradation of [^{14}C,^{3}H]**1** suggest that brefeldin A was uniformly labeled at the even-numbered positions by the biosynthetic incorporation of [^{14}C,^{3}H]-acetate. However, the value of the results is compromised by the inability to determine conveniently the ^{3}H content at each labeled site of **1**. The use of acetate labeled with stable isotopes combined with an NMR analysis of **1** labeled with [^{13}C,^{2}H]acetate is not subject to such experimental limitations.

Scheme I[a]



[a] (a) PyrH^{+}CrO$_3$Cl^{-}, CH$_2$Cl$_2$. (b) *t*-BuOK, *t*-BuOD, 25 °C, 20 h; done twice. (c) CrO$_3$, H^{+}, Δ. (d) MEM chloride, Et(*i*-Pr)$_2$N, 25 °C. (e) PyrH^{+}CrO$_3$Cl^{-}, CH$_2$Cl$_2$. (f) NaBH$_4$, EtOH, −78 °C. (g) TiCl$_4$, CH$_2$Cl$_2$, 25 °C, 20 min.

A sample of brefeldin A, which had been obtained from the fermentation of *P. brefeldianum* Dodge (Sandoz 464) with [2-^{13}C,2-^{2}H$_3$]acetate for 3 days, was converted to its 4,7-diacetate (**6**) to increase the solubility in C^2HCl_3. Analysis of the NOE-suppressed ^{13}C{^{1}H,^{2}H} NMR spectrum of **6** (Figure 1) gave ^{2}H and ^{13}C isotopic labeling data shown in Table I. The fraction of ^{13}C–^{2}H bonds which survived biochemical and isotopic exchange processes (F_D) at the even-numbered positions of [^{13}C,^{2}H]**6** are calculated by the method outlined in a footnote to Table I.

These data reveal important new information concerning the regiochemical course of isotopic hydrogen labeling of brefeldin A by acetate. Differences in ^{2}H enrichment are observed among

(9) By suitable adaptation of the procedures described by: Corey, E. J.; Wollenberg, R. H. *Tetrahedron Lett.* **1976**, 4701–4704.

(10) The ^{3}H/^{14}C ratio of **4** (determined as its *p*-phenylphenacyl derivative) was 16% less than that of the precursor acetate. This result may be due to partial loss of ^{3}H label due to exchange during the Kuhn–Roth oxidation or the derivation of a small amount of starter unit acetate from malonate by reversal of the acetate-to-malonate carboxylation process in vivo. We favor the latter interpretation on the basis of our data obtained from the incorporation of $^{13}C^2H_3CO_2H$ into brefeldin A.

Table I. Isotopic Enrichment of 4,7-Diacetylbrefeldin A (6) Labeled from [2-^{13}C,2-^{2}H$_3$]Acetate (90% ^{13}C, 98% ^{2}H)

carbon	δ_C[a]	$\Delta\delta_C$[a]	$100p_A$[b]	x_p[c]	$100p_p$[d]	F_R[e]	F_D, %[f]
2	118.64	0.28	3.4	0.0262	2.4	0.54 (^{13}CH), 0.46 (^{13}C^{2}H)	47
4	76.65	0.36	3.6	0.0283	2.6	0.55 (^{13}CH), 0.45 (^{13}C^{2}H)	46
6	38.69	0.32	3.9	0.0316	2.8	0.47 (^{13}CH), 0.53 (^{13}C^{2}H)	54
8	40.49	0.36	3.8	0.0299	2.7	0.56 (^{13}CH), 0.43 (^{13}C^{2}H)	44
10	136.31	0.38	3.3	0.0251	2.3	0.44 (^{13}CH), 0.56 (^{13}C^{2}H)	57
12	32.31	0.37	3.8	0.0307	2.8	0.91 (^{13}CH), 0.09 (^{13}C^{2}H)	9
14	34.51	0.46	3.9	0.0314	2.8	0.39 (^{13}CH$_2$), 0.26 (^{13}CH^2), 0.35 (^{13}C^{2}H$_2$)	49
		0.85					
16	20.91	0.28	3.5	0.0270	2.4	0.09 (^{13}CH$_3$), 0.09 (^{13}CH$_2$^{2}H), 0.21 (^{13}CH^{2}H$_2$)	87
		0.55					
		0.83				0.68 (^{13}C^{2}H$_3$)	
typical error	±0.03	±0.03	±0.03	0.03	±0.3	±0.01	±2

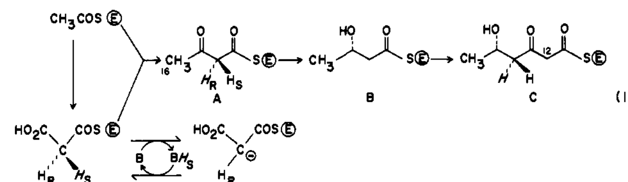
[a] δ_C referred to internal (CH$_3$)$_4$Si, solvent 4:1 C^{2}HCl$_3$/C$_6$F$_6$; $\Delta\delta_C$ = isotope chemical shift in ppm. [b] Average ^{13}C probability $p_A = 0.0111 I_T/I_M$, where I_T = total integrated intensity of the resonances for a labeled site, I_M = mean intensity for unenriched sites, and 0.0111 = natural abundance of ^{13}C. [c] Mole fraction of carbon originating from labeled precursor $x_p = (p_A - 0.0111)/(B - 0.0111)$ where $B = 0.90$ = probability of ^{13}C at C-2 of labeled precursor. [d] $p_p = Bx_p$ = ^{13}C probability at labeled sites in brefeldin A derived from the labeled precursor. [e] Fractions of ^{13}C bonded to ^{1}H and ^{2}H in brefeldin A derived from the labeled precursor, calculated from the intensities I of isotopically shifted and unshifted resonances after subtraction of the contribution to the latter due to ^{13}C from natural-abundance acetate; e.g., for C-14, $F_R(^{13}C^2H_2) = I(^{13}C^2H_2)/[I(^{13}C^2H_2) + I(^{13}C^2H^1H) + I(^{13}C^1H_2)]$. [f] F_D = percentage of the theoretical maximum of ^{2}H retained, e.g., for C-2 through C-12, $F_D = [100/0.98][I(^{13}C^2H)]/[I(^{13}C^2H) + I(^{13}C^1H)]$; for C-16, $F_D = [100/0.98][I(^{13}C^2H_3) + (^2/_3)I(^{13}C^1H^2H_2) + (^1/_3)I(^{13}C^1H_2{}^2H)]/[I(^{13}C^2H_3) + I(^{13}C^1H_2{}^2H) + I(^{13}C^1H_3)]$.

the eight even-numbered positions, whereas the ^{13}C enrichment is uniform within experimental error (Table I). The predominant species found at C-16 is ^{13}C^{2}H$_3$, with lesser amounts of ^{13}C^{1}H^{2}H$_2$, ^{13}C^{1}H$_2$^{2}H, and ^{13}C^{1}H$_3$ corresponding to a ^{2}H loss of only 13 ± 2%. The almost complete absence of ^{2}H at C-12 and the presence of ^{13}C^{2}H$_2$ in addition to ^{13}C^{1}H^{2}H and ^{13}C^{1}H$_2$ at C-14 are noteworthy.

The regiochemical distribution of ^{13}C and ^{2}H in labeled **1** allows important deductions to be made in comparing the biochemistry of fatty acids and macrolide antibiotics. Common features of the biosyntheses of **1** and fatty acids[8d] include equal ^{13}C enrichment at the even-numbered positions, C-2 through C-10 of **1**, that bear a single ^{2}H derived from dideuteriomalonate and the presence of ^{13}C^{2}H$_3$ which identifies the acetate starter group.

Sedgwick and Cornforth's studies with purified fatty acid synthetases from yeast and chicken liver have shown that 19–36% of the hydrogen isotope at carbons derived from chirally labeled malonate is lost by an exchange process exhibiting a kinetic isotope effect similar to that observed for enolizations.[5a,b] By the use of chiral substrates, they were able to restrict the possible mechanisms to stereospecific exchange with retention of configuration before the dehydrase step or (as they preferred) nonstereospecific exchange with epimerization at the β-methylene groups of the acyl-enzyme. Subsequently, McInnes et al.[8d] established that ^{2}H loss at corresponding carbons of palmitic acid enriched with [2-^{13}C,2-^{2}H$_3$]acetate by *Anacystis nidulans* was dependent on chain length, varying from 27% at C-2 to 37% at C-14. More recent studies by these workers on saturated and unsaturated fatty acids from yeast and two algae, however, have shown that the ^{2}H loss varies in amount, and with the chain length, in a characteristic way for each organism.[11] They also found evidence that could be interpreted as consistent with the isotopic exchange process being stereospecific for the pro-*S* hydrogen of malonyl-enzyme and occurring with retention of configuration. Stereospecific exchange at the β-ketoacyl-enzyme stage was excluded because Arnstadt et al.[12] had unequivocally demonstrated that no exchange occurred if NADPH was available for its reduction. As ^{2}H retention at C-2 through C-10 of **1** (see Table I) falls within the limits observed for fatty acids,[8d,11] it seems highly probable that the stereospecific exchange process described above is also the main route by which ^{2}H loss occurs during the formation of **1**.

Extensive loss of ^{2}H at C-12 of **1** can be rationalized in two ways. If reaction of malonyl- and 3-hydroxybutyryl-enzyme is slow enough to permit almost complete exchange of the deuterium at the pro-*S* position of the former and the pro-*R* deuterium is lost during a subsequent dehydration step, as in fatty acid biosynthesis,[5c] then very little ^{2}H may be retained at C-12. Significant retention of the pro-*S* deuterium of malonyl-enzyme would require additional exchange of the pro-*R* deuterium at the activated methylene group of 5-hydroxy-3-ketohexanoyl-enzyme (C, eq 1) in order to account for the nearly complete loss of ^{2}H at C-12.



This follows if condensation of acyl- and malonyl-enzyme proceeds (→ A, eq 1) with inversion of configuration as is the case for fatty acids.[5d] Thus deuterium remaining at the pro-*S* position of malonyl-enzyme will have a pro-*R* configuration at C-2 of A. Removal of the latter by another exchange process having a different stereospecific requirement seems unlikely as the activated methylene groups of malonyl-enzyme and C would be expected to experience a similar environment on the enzyme surface. Alternatively, the loss of ^{2}H from C-12 may be due to its stereospecific removal at some stage of the biosynthetic pathway subsequent to the steps of the carbon chain elongation process (eq 1). Further experiments are necessary before we can decide between these two possibilities.

The distribution of ^{13}C^{1}H$_2$, ^{13}C^{1}H^{2}H, and ^{13}C^{2}H$_2$ at C-14 of **1** cannot be explained at present. Nevertheless, the presence of ^{13}C^{2}H$_2$ suggests that NADPH was available for the immediate reduction of acetoacetyl-enzyme[12] (→ B, eq 1) and provides final confirmation that the macrolide is not formed from a fatty acid,[1,13] as this grouping has no counterpart in fatty acid biosynthesis. If initial ^{2}H loss occurred at the malonyl-enzyme stage, as suggested above, most of the ^{2}H in the ^{13}C^{1}H^{2}H groups would be expected to have a pro-*S* configuration. Attempts to confirm this by ^{2}H NMR were unsuccessful because of inadequate chemical shift dispersion.

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(13) Cross, B. E.; Hendley, P. *J. Chem. Soc., Chem. Commun.* **1975**, 124–125.

of Pharmacy, University of Wisconsin, for spectral determinations, Luigi Garlaschelli for preparation of **1** labeled by [1-^{14}C,2-^{3}H]-acetate, and Professor J. W. Cornforth (Sussex, England) for critical reading of the manuscript.

Biosynthesis of Macrolide Antibiotics. 4.[1] Stereochemistry of Hydrogen Labeling of Brefeldin A by [2-^{2}H$_3$]Acetate[†]

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Macrolide antibiotics are some of the structurally and stereochemically most diverse natural products known to science. Apart from the knowledge that these antibiotics are assembled from simple two-to-four carbon compounds, little is known about their biosynthetic pathways.[2,3] Nevertheless, it is usually presumed that macrolides are assembled in a manner analogous to the well-understood biosynthesis of fatty acids.[3]

We are testing the above assumption by examining the biosynthesis of selected macrolides. In the preceding paper,[1] we have

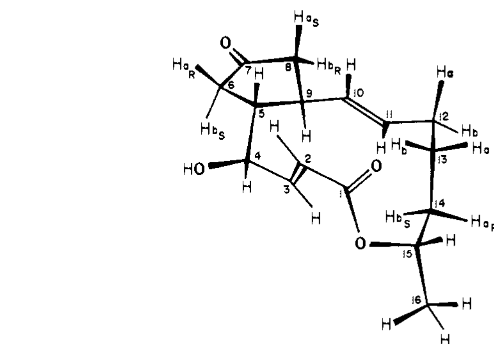

Figure 1. Conformation of 7-oxobrefeldin A (3), 7 mg, dissolved in degassed pyridine-d_5, 0.3 mL, that is consistent with qualitative intramolecular proton relationships deduced from ^{1}H:^{1}H nuclear Overhauser enhancement difference spectroscopy experiments.

summarized our observations on the regiochemistry of isotopic hydrogen labeling of the C_{16} macrolide antibiotic, brefeldin A (**1**), by acetate in vivo. In the present paper we describe the stereochemistry of this labeling process and consider the comparative biochemistry of fatty acids and macrolide antibiotics.

Examination of the regiochemical distribution of isotopic labels in brefeldin A enriched with [2-^{3}H]- and [2-^{2}H$_3$]acetate[1] has revealed that C-6 and C-8 are the only methylene groups isotopically substituted in the same way as the corresponding groups in fatty acids. Configurational assignments at these positions therefore permit the stereochemistry of labeling of **1** to be compared directly with that observed for fatty acids. If introduction of labels has proceeded by the stereospecific reduction of enoyl-enzyme intermediates, as occurs in the biosynthesis of fatty acids (vide infra), either the pro-*R* or pro-*S* diastereotopic hydrogens attached to C-6 and C-8 will be isotopically substituted.

The labeling stereochemistry was determined by first assigning the ^{1}H NMR spectrum of a suitable derivative of **1** and then analyzing the ^{2}H NMR spectrum of the same derivative prepared from **1** enriched with [2-^{2}H$_3$]acetate. 4,7-Diacetylbrefeldin A (**2**) was used for some preliminary experiments; however, 7-oxobrefeldin A (**3**) proved to be the only derivative with a large enough

Table I. ^{1}H NMR Spectral Data for 7-Oxobrefeldin A (**3**)[a]

b	H-2	H-3	H-4	H-5	H-6a	H-6b	
c	6.56	7.71	4.48	2.33	3.13	2.52	
d	dd	dd	ddd	dddd	ddd	ddd	
e	$J_{2,3} = 15.5$ $J_{2,4} = 2.0$	$J_{3,4} = 3.3$	$J_{4,5} = 9.4$	$J_{5,6a} = 7.9$ $J_{5,6b} = 10.4$ $J_{5,9} = 9.7$	$J_{6a,6b} = 18.1$ $J_{6a,8a} = 1.4$	$J_{6b,8b} = 1.4$	
b	H-8a	H-8b	H-9	H-10	H-11	H-12a	
c	2.25	2.62	2.81	2.25	5.84	1.98	
d	ddd	ddd	dddd	dd	ddd	dddd	
e	$J_{8a,8b} = 18.1$ $J_{8a,9} = 11.1$	$J_{8b,9} = 8.1$	$J_{9,10} = 9.1$	$J_{10,11} = 15.3$	$J_{11,12a} = 10.1$ $J_{11,12b} = 5.1$	$J_{12a,12b} = 12.5$ $J_{12a,13a} = 2.6$ $J_{12a,13b} = 5.1$	
b	H-12b	H-13a	H-13b	H-14a	H-14b	H-15	H-16
c	1.81	1.06	1.70	1.61	1.50	4.98	1.24
d	dddd	ddddd	dddd	ddd	dddd	m	d
e	$J_{12b,13a} = 12.2$ $J_{12b,13b} = 2.6$	$J_{13a,13b} = 14.3$ $J_{13a,14a} = 7.3$ $J_{13a,14b} = 1.3$	$J_{13b,14a} < 0.1$ $J_{13b,14b} = 6.1$	$J_{14a,14b} = 14.3$ $J_{14a,15} = 2.4$	$J_{14b,15} = 8.3$	$J_{15,16} = 6.25$	

[a] Determined at 270 MHz on a Bruker WH-270 NMR spectrometer. SW = 2400 Hz, T = 28 °C, resolution = ±0.08 Hz. Compound (5 mg) was dissolved in pyridine-d_5 (0.3 mL). [b] Numbering corresponds to numbering shown in Figure 1. [c] Chemical shifts are in ppm relative to internal Me_4Si as standard. [d] Signal multiplicity. [e] Coupling constants, $J_{p,q}$, are in Hz. All spin–spin coupling systems were analyzed with the Nicolet spin simulation program on ITRCAL 1 and 2 data systems.

chemical shift dispersion in the ^{2}H NMR spectrum for the easy identification of resonances due to diastereotopic deuteriums at C-6 and C-8.

^{1}H NMR assignments for **3** were checked by two independent techniques. Proton double irradiation experiments at 270 MHz in $C_5{}^2H_5N$, and at 360 MHz in C^2HCl_3 and $C_5{}^2H_5N$, combined

†NRCC No. 19056.

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