

surements; however, this hardly would have been possible in the present case, as explained previously. Furthermore, a wider temperature interval would introduce even more complex problems, due to the temperature dependence of the fitting parameters.

A solid convergence was obtained, even allowing all three parameters to vary freely. The final  $\epsilon_{CT}$  value ( $5520 (250) \text{ M}^{-1} \text{ cm}^{-1}$ ) was close to the average of the individual values obtained at different temperatures.

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## Biosynthesis of Macrolide Antibiotics. 6. Late Steps in Brefeldin A Biosynthesis

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**Abstract:** Brefeldin A (**1**), a macrolide antibiotic produced by several fungi, contains a cyclopentanol ring as part of its 16-membered lactone. The role that the oxygen and double bond functionality at positions 2, 3, 4, 7, 10, and 11 play in the mechanism of 5-membered ring formation is examined by comparing the efficiency of incorporation of several isotopically labeled compounds into **1** which are putative intermediates of the late part of the biosynthetic pathway. Brefeldin C (7-deoxybrefeldin A, **3**) and 7-oxobrefeldin A (**2**) were efficiently metabolized to **1**, 4-deoxybrefeldin C (**8**) was inefficiently metabolized to **1**, but the 3,4-double bond isomers of 4-deoxybrefeldin C and 2,3,10,11-tetrahydrobrefeldin C were not metabolized to **1** by intact cells of *Eupenicillium brefeldianum*. 7-Oxobrefeldin A was found in the fungal cells, and a small amount of exogenously added brefeldin A was converted to **2** in vivo. These results support the intermediacy of **3** in the biosynthetic pathway of **1** and suggest that **1** and **2** can be interconverted by a biochemical redox process. The low conversion of **8** to **1**, however, suggests that it is not an intermediate of the main biosynthetic pathway to **1** but possibly only metabolized to **1** by a shunt pathway or by a nonspecific hydroxylase.

Brefeldin A (**1**), a macrolide antibiotic produced by several fungi,<sup>1</sup> resembles the prostaglandins structurally, and for this reason, its biosynthesis has attracted considerable interest.<sup>1</sup> The central issue is the timing and mechanism of the formation of its cyclopentanol ring. Does this occur before or after the closure of the larger macrolide ring, and does the functionality at positions 2, 3, 4, 7, 10, and 11 have a role in the cyclization process? From the results of isotopic labeling experiments with <sup>18</sup>O-<sup>1</sup> and <sup>2</sup>H-labeled<sup>2-4</sup> acetate, we have proposed that closure of a 16-membered macrolide generates the 5-membered ring of **1** by an epoxyolefin cyclization mechanism which would create the C-4 hydroxyl and C-10 olefin simultaneously.<sup>1</sup> Therefore, we needed to determine when these and the other functionality surrounding the 5-membered ring are introduced during the biosynthesis of **1**. The information presented below shows that in the main pathway to brefeldin A, introduction of the C-7 hydroxyl onto an existing cyclopentane ring is an efficient, terminal step but that the introduction of the C-4 hydroxyl and C-2 and C-10 double bonds is not. Furthermore, the C-7 functionality can interconvert between the hydroxyl and ketone oxidation states as an incidental part of the metabolism of **1**.

The isotopically labeled compounds to be tested as biosynthetic precursors of **1** were synthesized from brefeldin A as shown in Scheme I. The preparation of [7-<sup>2</sup>H,7-<sup>3</sup>H]brefeldin C (**3**) was adapted from the methods used by Sunagawa et al. in their work on the identification of **3**,<sup>5</sup> the preparation of [7-<sup>2</sup>H,7-<sup>3</sup>H]-2,3,10,11-tetrahydrobrefeldin C (**4**) from the method of Harri et al. for the reduction of **1**,<sup>6</sup> and the preparation of [4-<sup>2</sup>H,4-<sup>3</sup>H]-

brefeldin A and -7-oxobrefeldin A from the methods of Corey and Wollenberg.<sup>7</sup> The synthesis of 4-deoxybrefeldin C (**8**) was complicated by preferred isomerization of the 2,3-double bond into the unconjugated 3,4-position.<sup>8</sup> The most successful among several procedures we tried for C-4 deoxygenation<sup>9</sup> was an adaptation of the method of Keinan and Greenspoon<sup>10</sup> in which the addition of a catalytic amount of Pd(PPh<sub>3</sub>)<sub>4</sub> was found to promote formation of the desired 2,3-double bond isomer.<sup>11</sup> Deoxygenation of 7-oxobrefeldin A gave two isomeric products (**6** and **7**) in about equal yield which were converted into [7-<sup>3</sup>H]-**8** and its 3,4-double bond isomer **9** by the methods used for the preparation of [7-<sup>2</sup>H,7-<sup>3</sup>H]-**3**. All the isotopically labeled compounds were purified chromatographically and had the same physical and spectral characteristics as their unlabeled counterparts except, of course, for the absence of certain resonances in the proton NMR spectra of the corresponding deuterium-labeled compounds.

We examined the incorporation of each of these labeled compounds into brefeldin A by separate feeding experiments with *Eupenicillium brefeldianum* in which the putative precursor was fed on the third or fourth day of the fermentation period followed by three more days of growth. The isolated **1** was recrystallized to constant specific radioactivity and the site of its expected isotopic labeling confirmed by degradation or <sup>2</sup>H NMR spectroscopy. The

(7) Corey, E. J.; Wollenberg, R. H. *Tetrahedron Lett.* **1976**, 4701.

(8) The 3,4-double bond isomer is the exclusive product when the mixture of 2,3/3,4-double bond isomers are treated with *t*-BuOH/*t*-BuOH-Me<sub>2</sub>SO at room temperature or LDA/THF at -78 °C.

(9) We could not repeat the work of Entwistle, Howard, and Johnstone (Entwistle, I. D.; Howard, C. C.; Johnstone, R. A. W. *Phytochemistry* **1974**, *13*, 173) in which they prepared 4-deoxybrefeldin A in a 44% yield by Li/liquid NH<sub>3</sub> reduction of 4,7-diacetyl-brefeldin A.

(10) Keinan, E.; Greenspoon, N. *Tetrahedron Lett.* **1982**, 23, 241.

(11) The intermediate  $\pi$ -allylpalladium(0) complex presumably is formed in the reduction of 4,7-diacetyl-brefeldin A or 4-acetyl-7-oxobrefeldin A and then is reduced at C-2 or C-4 or eliminates AcOH to form a 2,3,4,5,10,11-triene derivative of brefeldin A.<sup>12</sup> We are not certain that **5** forms the same intermediate Pd(0) complex although unpublished results from Prof. Barry Trost's laboratory support this possibility.

(12) Tsuji, J.; Yamakawa, T. *Tetrahedron Lett.* **1979**, 613. Hutchins, R. O.; Learn, K.; Fulton, R. P. *Tetrahedron Lett.* **1980**, 27.

(1) Mabuni, C. T.; Garlaschelli, L.; Ellison, R. A.; Hutchinson, C. R. *J. Am. Chem. Soc.* **1979**, *101*, 707 and references therein.

(2) Hutchinson, C. R.; Kurobane, I.; Mabuni, C. T.; Kumola, R. W.; McInnes, A. G.; Walter, J. A. *J. Am. Chem. Soc.* **1981**, *103*, 2474.

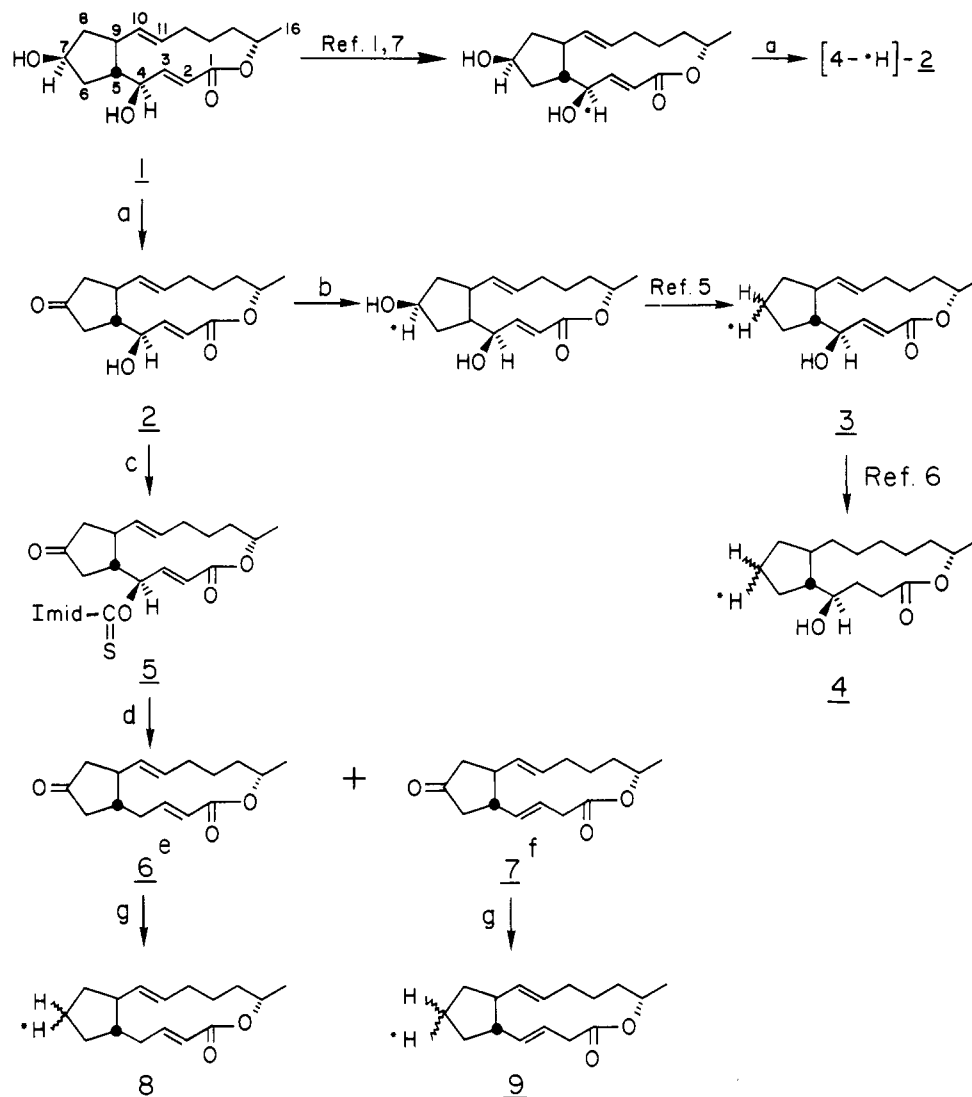
(3) Hutchinson, C. R.; Kurobane, I.; Cane, D. E.; Hasler, H.; McInnes, A. G. *J. Am. Chem. Soc.* **1981**, *103*, 2477.

(4) Hutchinson, C. R.; Shu-Wen, Li; McInnes, A. G.; Walter, J. A. *Tetrahedron* **1983**, *39*, 3507.

(5) Sunagawa, M.; Ohta, T.; Nozoe, S. *Heterocycles* **1979**, *13*, 267.

(6) Harri, E.; Loeffler, W.; Sigg, H. P.; Stahelin, H.; Tamm, C. *Helv. Chim. Acta* **1963**, *46*, 1235.

Scheme 1



<sup>a</sup> PCC,  $\text{CH}_2\text{Cl}_2$ , room temperature. <sup>b</sup>  $\text{NaBH}_4$ ,  $i\text{-PrOH}$ ,  $-20^\circ\text{C}$ . <sup>c</sup> (Imidazole) $_2\text{C}=\text{S}$ ,  $\text{CH}_2\text{Cl}_2$ , room temperature. <sup>d</sup>  $(n\text{-Bu})_3\text{SnH}$ ,  $\text{Pd}(\text{PPh}_3)_4$ , AIBN, toluene,  $75^\circ\text{C}$   $\rightarrow$  room temperature, 20 min. <sup>e</sup> 25% yield; mp  $112\text{--}113^\circ\text{C}$ . All chemical and spectral data were consistent with the assigned structure. <sup>f</sup> 31% yield; mp  $114\text{--}115^\circ\text{C}$ . All chemical and spectral data were consistent with the assigned structure. <sup>g</sup> Same sequence used for the labeling of  $[7\text{-}^2\text{H}, 7\text{-}^3\text{H}]\text{-3}$ .

**Table I.** Relative Efficiency of Incorporation of Isotopically Labeled Precursors into Brefeldin A (1)

expt	precursor	amt fed		amt 1 isolated, mg	incorporation, %	
		mg	$\mu\text{Ci}$		specific	tot
1	$[7\text{-}^2\text{H}, 7\text{-}^3\text{H}]\text{-3}$	0.93	5.8	16 (B <sup>b</sup> )	8.6	15
2	$[7\text{-}^3\text{H}, 4\text{-}^2\text{H}]\text{-3}^a$	20	12	21 (B); 18 (M) <sup>c</sup>	7.1 (B); 7.5 (M)	16 (B + M)
3	$[4\text{-}^3\text{H}]\text{-2} + [4\text{-}^2\text{H}]\text{-2}^d$	0.88	4.4			
4	$[4\text{-}^3\text{H}]\text{-1}$	2.1	10.5	85 (B); 64 (M) + 2, 2 (M) <sup>e</sup>	0.02	0.019
5	$[7\text{-}^3\text{H}]\text{-4}$	22	6.5	30 (B)	$\sim 0$	
6	$[7\text{-}^3\text{H}]\text{-8}$	0.5	126	30 (B); 25 (M)	0.015 (B); 0.008 (M)	0.80 (B)
7	$[7\text{-}^3\text{H}]\text{-8} + [7\text{-}^2\text{H}]\text{-3}^e, h$	10	2400	35 (B); 29 (M)	0.3 (B); 0.06 (M) <sup>f</sup>	0.94 (B)
8	$[7\text{-}^3\text{H}]\text{-9}$	25				
		3.1	100	28 (B); 35 (M)	$\sim 0$	

<sup>a</sup> 97 mol %  $d_1$ . <sup>b</sup> From the fermentation broth. <sup>c</sup> From the mycelia. <sup>d</sup>  $>95$  mol %  $d_1$ . <sup>e</sup> 42% of 1 was recovered. <sup>f</sup> 0.29% into 4-deoxy-1. <sup>g</sup> 95 mol %  $d_1$ . <sup>h</sup> This was the positive control for the feeding experiment.

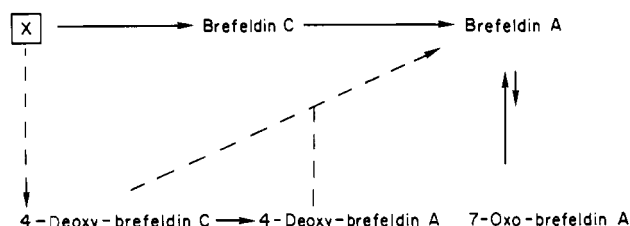
results of these feeding experiments are shown in Table I.

Comparison of the relative incorporations of the compounds whose metabolism we studied reveals that only brefeldin C (experiments 1 and 2) and 7-oxobrefeldin-A (experiment 3) were efficiently converted to brefeldin A. Oxidation of the  $[7\text{-}^2\text{H}, 7\text{-}^3\text{H}]\text{-1}$  from experiment 1 resulted in loss of  $>99\%$  of the tritium labeling;  $^2\text{H}$  NMR analysis of the  $[4\text{-}^2\text{H}]\text{-1}$  from experiments 2 and 3 showed that deuterium was located only at C-4. Sunagawa

et al. have reported a corresponding result for 3,<sup>13</sup> which is consistent with the presence of 3 in *Eupenicillium brefeldianum*.<sup>5</sup>

(13) Sunagawa, M.; Ohta, T.; Nozoe, S. *J. Antibiot.* **1983**, *36*, 25.

(14) Since the two diastereotopic positions at C-7 presumably were randomly labeled with  $^3\text{H}$ , some  $^3\text{H}$  necessarily was removed during the conversion of  $[7\text{-}^3\text{H}]\text{-8}$  to  $[7\text{-}^3\text{H}]\text{-1}$ . This  $^3\text{H}$  could have entered pools of intermediary coenzymes and metabolites sufficient to label 1 at positions other than C-7. Note that a large amount of radioactivity was fed in experiment 7.



**Figure 1.** Solid lines indicate metabolic steps in the main biosynthetic pathway to brefeldin A and the dashed lines steps in shunt pathways or steps catalyzed by nonspecific enzymes. "X" is the unknown intermediate made from eight acetate-derived  $C_2$  units in which only the 16-membered ring has been closed.

We isolated **2** from this fungus and confirmed its structure by comparison with an authentic standard made from **1**,<sup>6,7,17</sup> however, its presence and efficient reduction to **1** in vivo do not by themselves justify its direct involvement in the biosynthesis of **1**. It is logical from the viewpoint of metabolic efficiency to assume that the metabolic sequence  $3 \rightarrow 1 \rightarrow 2$  would take place in vivo rather than the alternative,  $3 \rightarrow 2 \rightarrow 1$ . We also are not aware of any example in secondary metabolism where a saturated carbon is directly oxidized to a carbonyl group. Therefore, we believe that the major biochemical pathway is conversion of brefeldin C directly to brefeldin A, yet a small amount of brefeldin A can be oxidized to 7-oxobrefeldin A (experiment 4) even though the reverse direction of this redox process clearly is favored (experiment 3). We cannot rule out the possibility, however, that the reduction of **2** was catalyzed by an oxidoreductase that normally has no role in brefeldin A biosynthesis.

The relative incorporation efficiencies of 2,3,10,11-tetrahydrobrefeldin C (**4**, experiment 5), 4-deoxybrefeldin C (**8**, experiments 6 and 7), and its 3,4-double bond isomer (**9**, experiment 8) given in Table I show that none of these compounds were metabolized to brefeldin A as efficiently as the 7-deoxy or 7-oxo derivatives. The data rule out the introduction of the double bonds at positions 2 and 10 as late events in the biosynthesis of brefeldin A since any brefeldin C formed by desaturation of the tetrahydro precursor should have been metabolized to brefeldin A. The structural similarity between **3** and **4** should discount differences in uptake by the fungal cells as being the reason for this negative result, but we did not eliminate this possibility by direct experimentation. By this same argument, we feel that the negative result from the feeding experiment with **9** shows that it cannot be metabolized to brefeldin A via brefeldin C. On the other hand, [ $7\text{-}^3\text{H}$ ]-**8** was incorporated into brefeldin A with a low efficiency, and oxidation of a sample of [ $^2\text{H},^3\text{H}$ ]-**1** from experiment 7 to **2** resulted in loss of only 80% of the tritium labeling.<sup>14</sup> A very small amount of an unknown, radioactive compound whose TLC behavior in two solvent systems was identical with an authentic sample of 4-deoxybrefeldin A also was isolated from this feeding experiment. Therefore, we believe that 4-deoxybrefeldin C is metabolized to brefeldin C and thence to brefeldin A in vivo but that the rate of C-4 hydroxylation is slow enough for some 4-deoxybrefeldin A to accumulate. (We could not detect 4-deoxybrefeldin A in any of the other feeding experiments.) This observation and the lower incorporation of **8** than **2** or **3** into **1** leads us to propose that 4-deoxybrefeldin C is not an intermediate of the main biosynthetic pathway to brefeldin A.<sup>15</sup>

The results obtained from our feeding experiments are consistent with the metabolic gridwork shown in Figure 1 for the stage in brefeldin A biosynthesis that follows formation of the 5- and

16-membered rings. Because of the imprecise nature of metabolic mapping experiments done with whole cells, there are limitations on what we can conclude from our incorporation efficiency data. Nevertheless, it appears that introduction of the double bonds at positions 2 and 10 precedes formation of the 5-membered ring and that brefeldin C precedes brefeldin A in the biosynthetic pathway. 7-Oxobrefeldin A is probably a catabolite of brefeldin A with its preferred reduction to **1** (or perhaps to 7-epi-**1**)<sup>16</sup> being part of the reason that brefeldin A is the principal macrolide accumulated by the fungus. The data do not prove beyond doubt that 4-deoxybrefeldin C is an intermediate of the main biosynthetic pathway; its metabolism to **1** could be only a shunt process or even due to the action of a nonspecific C-4 hydroxylase. Therefore, our hypothesis<sup>1</sup> that formation of the 5-membered ring of brefeldin C directly introduces the oxygen at C-4 is not disproven by the present data even though it will be prudent to test alternative mechanisms in future experiments. For this reason we designate the immediate precursor of cyclopentane formation as "X" in Figure 1 since its structure remains elusive.

## Experimental Section

**4-Thiocarbonylimidazolite of 7-Oxobrefeldin A (5).** Thiocarbonylimidazole (385 mg, 2.16 mmol) was added to a solution of 7-oxobrefeldin A<sup>7</sup> (200 mg, 0.72 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (2 mL) at  $0^\circ\text{C}$ , and the reaction mixture was allowed to warm to room temperature. After 2.5 h, additional thiocarbonylimidazole (90 mg) was added to the reaction and the mixture stirred for an additional 30 min.  $\text{CH}_2\text{Cl}_2$  (10 mL) and 1 N HCl (10 mL) were added to quench the reaction and then the organic layer was separated and washed with water then saturated aqueous  $\text{NaHCO}_3$ , dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and the solvent evaporated in vacuo to give an oil (quant yield). This material showed a single spot by TLC on silica gel in  $\text{CHCl}_3/\text{MeOH}$  (10:1) and was used without further purification for the next reaction:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 90 MHz)  $\delta$  1.26 (3 H, d,  $J = 6$  Hz), 1.40–2.90 (12 H, m), 5.00 (1 H, m), 7.10 (1 H, d,  $J = 1$  Hz), 7.28 (1 H, dd,  $J = 4, 15$  Hz), 7.85 (1 H, d,  $J = 1$  Hz), and 8.40 (1 H, s);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 22.5 MHz)  $\delta$  20.6, 26.1, 31.7, 34.3, 42.7, 43.8, 46.2, 47.5, 72.1, 85.5, 117.8, 120.5, 133.3, 134.3, 136.9, 143.3, 143.3, 164.8, and 213.4 (C=S resonance too weak to detect); MS did not show molecular ion.

**4-Deoxy-7-oxobrefeldin A (6) and Its Isomer (7).** A solution of (*n*-Bu)<sub>3</sub>SnH (230 mg, 0.7 mmol) in toluene was added dropwise to a suspension of **5** (270 mg, 0.7 mmol),  $\text{Pd}(\text{PPh}_3)_4$  (8 mg, 7  $\mu\text{mol}$ ), and a trace amount of AIBN in dry toluene (5 mL) at  $75^\circ\text{C}$  under a nitrogen atmosphere and the reaction mixture stirred for 10 min. Additional (*n*-Bu)<sub>3</sub>SnH (230 mg, 0.7 mmol) was added to the reaction, and the mixture was allowed to cool to room temperature and then stirred for an additional 10 min. The reaction mixture was filtered, the filtrate evaporated in vacuo, and the resulting crude residue purified by column chromatography on silica gel by using *n*-hexane/ethyl acetate (4:1) as the eluant to give **6** (46 mg, 25%) and **7** (56 mg, 31%).

**6:** mp  $112\text{--}113^\circ\text{C}$  (recrystallized from  $\text{MeOH-H}_2\text{O}$ ); UV (MeOH), 202 ( $\epsilon$  2490), and ca. 230 (sh) nm;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 90 MHz)  $\delta$  1.26 (3 H, d,  $J = 6.4$  Hz), 1.56–2.82 (12 H, m), 4.80 (1 H, m), 5.21 (1 H, dd,  $J = 8.3, 15.3$  Hz), 5.71 (1 H, d,  $J = 15.4$  Hz), 5.82 (1 H, m), and 7.30 (1 H, dd,  $J = 5.1, 11, 15.4$  Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 22.5 MHz)  $\delta$  20.8, 26.2, 31.8, 34.9, 39.1, 43.2, 46.3, 46.6, 47.2, 71.7, 122.0, 131.9, 136.1, 148.6, 166.5, and 210.0; MS,  $m/z$  (% rel intensity) 262.1570 calcd, 262.1560 found (5,  $\text{M}^+$ ), 220 (35), 164 (9), 134 (16), 95 (44), 80 (100), 67 (60), and 41 (66).

**7:** mp  $114\text{--}115^\circ\text{C}$  (recrystallized from  $\text{MeOH-H}_2\text{O}$ ); UV (MeOH), 202 ( $\epsilon$  2079) nm;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 90 MHz)  $\delta$  1.22 (3 H, d,  $J = 6.4$  Hz), 1.58–2.50 (12 H, m), 3.00 (2 H, d,  $J = 8$  Hz), 4.85 (1 H, m), 5.15 (1 H, dd,  $J = 8, 15.2$  Hz), 5.35–5.48 (2 H, m), and 5.70 (1 H, m);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 22.5 MHz)  $\delta$  20.7, 27.5, 31.2, 33.8, 40.5, 45.1 (two resonances), 47.3, 47.6, 71.5, 125.4, 131, 133.7, 135.6, 166.7, and 210.4; MS,  $m/z$  (% rel intensity) 262.1570 calcd, 262.1572 found (5,  $\text{M}^+$ ), 220 (11), 162 (3), 122 (12), 80 (100), 67 (58), and 41 (27).

**4-Deoxybrefeldin C (8) and Its Isomer (9).** Solid  $\text{NaBH}_4$  (13 mg, 0.34 mmol) was added slowly to a solution of **6** (44 mg, 0.1 mmol) in *i*-PrOH (1 mL) with magnetic stirring under a nitrogen atmosphere at room temperature. After 1.5 h, the excess  $\text{NaBH}_4$  was destroyed by the addition of AcOH (0.2 mL) at  $-20^\circ\text{C}$ , and the reaction mixture was evaporated to dryness in vacuo. The resulting crude residue was purified by column chromatography on silica gel with a  $\text{CHCl}_3$  eluant to give material showing a single spot on TLC (silica gel, *n*-hexane/ethyl acetate (1:1)). This was dissolved in dry pyridine (0.5 mL), cooled to  $-15^\circ\text{C}$  under a nitrogen atmosphere, and mesyl chloride (68 mg, 0.6 mmol) mixed with the solution. After 2 h, the reaction mixture was allowed to

(15) The scattering of tritium label in **1** found in experiment 7 but not in experiment 1 (but since much less radioactivity was fed in experiment 1, any scattering may have been below the detectable limits) also may indicate that **8** had undergone some amount of nonspecific metabolism. This possibility would support our conclusion that **8** is not an intermediate of the main biosynthetic pathway to **1**.

(16) 7-Epibrefeldin A has been found in *Curvularia lunata*: Gorst-Allman, C. P.; Steyn, P. S.; Rabie, C. J. *J. Chem. Soc., Perkin Trans. 1* **1982**, 2387.

(17) Macrolides **1** and **2** also have been found in *Alternaria carthami*: Tietjen, K. G.; Schaller, E.; Matern, U. *Physiol. Plant Pathol.* **1983**, 23, 387.

warm to room temperature and filtered and the filtrate evaporated in vacuo by a benzene azeotrope. The crude product was purified by column chromatography on silica gel with a  $\text{CHCl}_3$  eluant to give the pure mesylate. This material (54 mg, 0.16 mmol) was dissolved in dry glyme (1 mL), NaI (112 mg, 0.75 mmol) and Zn (98 mg, 1.5 mmol) were added, and the reaction mixture was stirred under a nitrogen atmosphere at 65 °C for 1.5 h. Then the mixture was filtered and the filtrate evaporated to dryness in vacuo. The residue was purified by column chromatography on silica gel by using *n*-hexane and then *n*-hexane/ethyl acetate (99:1) as eluants to give material that was crystallized from MeOH- $\text{H}_2\text{O}$  to give **8** (18 mg, 43% from **6**): mp 94-98 °C; UV (MeOH), 202 ( $\epsilon$  10904) nm;  $^1\text{H}$  NMR ( $\text{CD}_2\text{Cl}_2$ , 90 MHz)  $\delta$  0.95 (1 H, m), 1.23 (3 H, d,  $J$  = 6.5 Hz), 1.50-2.60 (15 H, m), 4.85 (1 H, m), 5.21 (1 H, dd,  $J$  = 10, 15 Hz), 5.63 (1 H, d,  $J$  = 15.6 Hz), 5.74 (1 H, m), and 7.33 (1 H, dd,  $J$  = 5, 11.9, 15.6 Hz);  $^{13}\text{C}$  NMR ( $\text{CD}_2\text{Cl}_2$ , 22.5 MHz)  $\delta$  21, 25.6, 27, 32.3, 34.9, 35.1, 35.7, 40, 47.6, 50.3, 71.7, 120.8, 130, 138, 151.1, and 166.8; MS,  $m/z$  (% rel intensity) 248.1777 calcd, 248.177 found (20,  $\text{M}^+$ ), 206 (37), 161 (22), 152 (39), 121 (57), 79 (83), and 40 (100).

The isomer **9** was prepared from **7** by using the same procedures: colorless oil; UV (MeOH), 201 ( $\epsilon$  3280) nm;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 90 MHz)  $\delta$  1.18 (3 H, d,  $J$  = 6.5 Hz), 1.30-2.60 (14 H, m), 2.98 (2 H, d,  $J$  = 8 Hz), 4.81 (1 H, m), and 5.00-5.68 (4 H, m);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 22.5 MHz)  $\delta$  20.6, 23.4, 28, 31.3, 32.2 (2 resonances), 33.9, 40.7, 51.2, 51.5, 71.3, 123.2, 131.5, 133.7, 138.5, and 166.7; MS,  $m/z$  (% rel intensity) 248 (2,  $\text{M}^+$ ), 206 (10), 152 (20), 121 (35), 120 (35), 80 (77), and 40 (100).

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## Synthetic Studies toward Aflavinine: A Synthesis of 3-Desmethylaflavinine via a [2 + 2 + 2] Annulation

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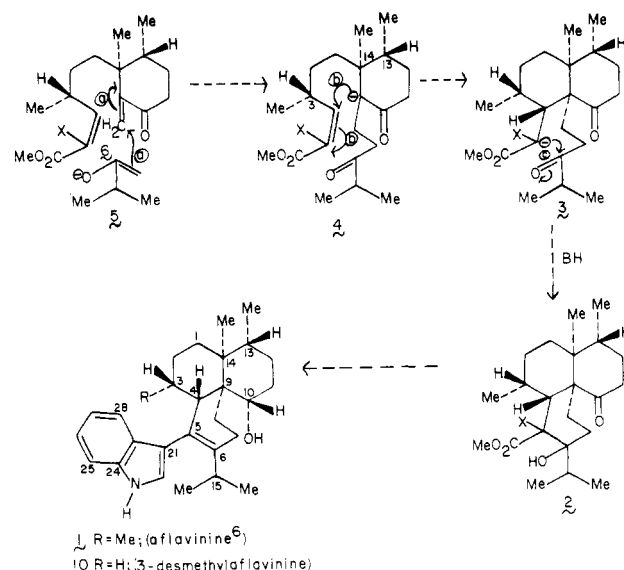
**Abstract:** Several systems containing two electrophilic olefins have been synthesized. In each case, one of the olefins was an  $\alpha$ -methylene ketone while the other was an  $\alpha,\beta$ -unsaturated ester. In each case the  $\alpha$ -methylene ketone was attacked by the external nucleophile which was the regiospecific lithium enolate derived from 2-(trimethylsiloxy)-3-methylbut-1-ene. Michael addition to the  $\beta$ -carbon of the  $\alpha$ -methylene ketone was followed by intramolecular Michael addition to the enoate. The ester enolate thus produced reacted in an intramolecular aldol-like reaction with the isopropyl ketone to complete the closure of a six-membered ring from the three  $\text{C}_2$  units (see Scheme I). The stereochemistry of this process has been investigated in some detail, and an application to the synthesis of 3-desmethylaflavinine (**10**) is described.

### Background

Aflavinine (**1**) is a structurally novel indolic diterpenoid which was isolated from a strain of *Aspergillus flavus*.<sup>1</sup> Other structurally related indolic diterpenoids, such as aflatrem, paspalinine, paspaline, paspalicine, and paaxiline have tremorogenic properties.<sup>2,3</sup> The small amount of homogeneous aflavinine which was isolated was apparently not subjected to biological evaluation. Hence, there is no reliable information on its neurological activity, if any.<sup>4</sup>

There exist no data in the literature pertaining to the chemical behavior of aflavinine. The information content of the spectroscopic measurements on aflavinine was too fragmentary to encourage even a meaningful hypothesis as to its structure and stereochemistry. It was to the province of X-ray crystallography that one had to turn for the structural elucidation.<sup>1</sup> Not unlike the case with many of the newer natural products available from natural sources in grudgingly small quantities, a plan for the total synthesis of aflavinine (**1**) would have to make do without the

Scheme I



(1) Gallagher, R. T.; McCabe, T.; Hirotsu, K.; Clardy, J.; Nicholson, J.; Wilson, B. J. *Tetrahedron Lett.* **1980**, 21, 243.

(2) Gallagher, R. T.; Clardy, J.; Wilson, B. J. *Tetrahedron Lett.* **1980**, 21, 239.

(3) (a) Gallagher, R. T.; Finer, J.; Clardy, J.; Leutwiler, A.; Weibel, F.; Werner, A.; Arigoni, D. *Tetrahedron Lett.* **1980**, 21, 235. (b) Springer, J. P.; Clardy, J. *Tetrahedron Lett.* **1980**, 21, 231. (c) Cole, R. J.; Kirksey, J. W.; Wells, J. M. *Can. J. Microbiol.* **1974**, 20, 1159.

(4) For a report on the biological activity of a hydroxylated aflavinine see: Cole, R. J.; Dorner, J. W.; Springer, J. P.; Cox, R. H. *J. Agric. Food Chem.* **1981**, 29, 293.

benefit of any information as to the chemical "personality" of its final target.

### Synthetic Rationale

Our interest in such a total synthesis arose from a perception that the basic ring system could be assembled from an intriguing