Fusidienol A: A Novel Ras Farnesyl-Protein Transferase Inhibitor from *Phoma* sp.

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Human tumorogenesis is a multifunctional process encompassing changes in the activity of multiple genes and their cognate proteins. One such gene product is Ras protein (p21). Post-translational modifications such as farnesylation, proteolysis, carboxymethylation, and palmitoylation are required for cell-transforming activity.[1] Ras (p21) proteins are farnesylated by farnesyl-protein transferase (FPTase) on the cysteine residue of the C-terminal CAAX box motif. Colon and pancreatic cancers,[2] where the *ras* oncogene is mutated and believed to play a major role in the formation of malignant tumors, could be potentially treated by selective FPTase inhibition. The biological efficacy of FPTase inhibitors as anticancer agents has been recently demonstrated using cell-based *ras* dependent transformation[3] and animal models.[4]

In last few years we have reported the isolation of selective novel FPTase inhibitors from microbial sources. These include chaetomellic acids,[5] actinoplanic acids,[6] preussomerins,[7] cylindrols,[8] barceloneic acid,[9] fusidienol,[10] and oreganic acid.[11] Other inhibitors have been reported by several groups and include pepticinnamins,[12] gliotoxin,[13] 10′-desmethoxystreptonigrin,[14] and manumycin analogs.[15] Our continued interest in finding potent and novel inhibitors of FPTase led to the isolation of fusidienol A (**1a**) from *Phoma* sps. Fusidienol A is the second member of the fusidienol (**1b**) family of inhibitors that possess a novel tricyclic oxygen-containing heterocycle with a 7/6/6 ring system. Like fusidienol, **1a** specifically inhibits recombinant human FPTase[16] with an IC_{50} value of 1.8 μM and is inactive against bovine brain geranyl-geranyl protein transferase (IC_{50} >50 μM).

The structure of fusidienol (**1b**) was elucidated by 2D NMR methods with the extensive use of HMBC correlations. However, there were uncertainties due to lack of the direct correlation of the B-ring γ-pyrone carbonyl and a single correlation to C-9 and C-15. The lability of fusidienol in organic solvents precluded its crystallization for X-ray crystallographic studies. In contrast, fusidienol A is stable in organic solvents. The major difference between the two compounds is the presence of an enylic hydroxymethyl group in fusidienol (**1b**) that may be responsible for the instability. The stability of the new compound, fusidienol A (**1a**), presented an opportunity for crystallization and confirmation of the structure by X-ray crystallography. Isolation, structure elucidation by NMR and X-ray crystallographic methods, mass spectral fragmentation, and biological activity of fusidienol A are the subject of discussion of the present report.

Isolation

An unidentified species of the fungal genus *Phoma* (MF 6118), isolated from a vegetation sample collected at

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(1) (a) Gibbs, J. B. *Semin. Can. Biol.* **1992**, *3*, 383. (b) Gibbs, J. B. *Cell* **1991**, *65*, 1 and references cited therein. (c) Der, C. J. and Cox, A. D. *Cancer Cell* **1991**, *3*, 331. (d) Reiss, Y.; Goldstein, J. L.; Seabra, M. C.; Casey, P. J.; Brown, M. S. *Cells* **1990**, *62*, 81. (e) Schaber, M. D.; O'Hara, M. B.; Garsky, V. M.; Mosser, S. D.; Bergstrom, J. D.; Moores, S. L.; Marshall, M. S.; Friedman, P. A.; Dixon, R. A. F.; Gibbs, J. B. *J. Biol. Chem.* **1990**, *265*, 14701. (f) Gibbs, J. B.; Pompliano, D. L.; Mosser, S. D.; Rands, E.; Lingham, R. B.; Singh, S. B.; Scolnick, E. M.; Kohl, N. E.; Oliff, A. *J. Biol. Chem.* **1993**, *268*, 7617. (g) Zhang, F. L.; Casey, P. J. *Annu. Rev. Biochem.* **1996**, *65*, 241. (h) Gibbs, J. B.; Oliff, A. *Annu. Rev. Pharmacol. Toxicol.* **1997**, *37*, 143. (I) Ayral-Kalousrian, S.; Skotnicki, J. S. *Annu. Rep. Med. Chem.* **1996**, *31*, 171.

(2) (a) Barbacid, M. *Annu. Rev. Biochem.* **1987**, *56*, 779. (b) Rodenhuis, S. *Semin. Can. Biol.* **1992**, *3*, 241.

(3) (a) Kohl, N. E.; Mosser, S. D.; deSolms, J. S.; Giuliani, E. A.; Pompliano, D. L.; Graham, S. L.; Smith, R. L.; Scolnick, E. M.; Oliff, A.; Gibbs, J. B. *Science* **1993**, *260*, 1934. (b) James, G. J.; Goldstein, J. L.; Brown, M. S.; Rawson, T. E.; Somers, T. C.; McDowell, R. S.; Crowley, C. W.; Lucas, B. K.; Levinson, A. D.; Marsters, J. C. *Science* **1993**, *260*, 1937.

(4) Kohl, N. E.; Wilson, F. R.; Mosser, S. D.; Giuliani, E.; DeSolms, S. J.; Conner, M. W.; Anthony, N. J.; Holtz, W. J.; Gomez, R. P.; Lee, T.-J.; Smith, R. L.; Graham, S. L.; Hartman, G. D.; Gibbs, J. B.; Oliff, A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 9141.

(5) (a) Singh, S. B.; Zink, D. L.; Liesch, J. M.; Goetz, M. A.; Jenkins, R. G.; Nallin-Omstead, M.; Silverman, K. C.; Bills, G. F.; Mosley, R. T.; Gibbs, J. B.; Albers-Schonberg, G.; Lingham, R. B. *Tetrahedron* **1993**, *49*, 5917. (b) Reference 1f. (c) Singh, S. B. *Tetrhedron Lett.* **1993**, *34*, 6521. (d) Lingham, R. B.; Silverman, K. C.; Bills, G. F.; Cascales, C.; Sanchez, M.; Jenkins, R. G.; Gartner, S.; Martin, I.; Pelaez, F.; Mochales, S.; Kong, L.; Burg, R. W.; Meinz, M. S.; Huang, L.; Nallin-Omstead, M.; Mosser, S. D.; Schaber, M. D.; Omer, C. A.; Pompliano, D. L.; Gibbs, J. B.; Singh, S. B. *Appl. Microbiol. Biotech.* **1993**, *40*, 370.

(6) (a) Singh, S. B. ; Liesch, J. M.; Lingham, R. B.; Goetz, M. A.; Gibbs, J. B. *J. Am. Chem. Soc.* **1994**, *116*, 11606. (b) Singh, S. B. ; Liesch, J. M.; Lingham, R. B.; Silverman, K. C.; Sigmund, J.; Goetz, M. A. *J. Org. Chem.* **1995**, *60*, 7896.

(7) Singh, S. B.; Zink, D. L.; Liesch, J. M. ; Ball, R. G.; Goetz, M. A.; Bolessa, E. A.; Giacobbe, R. A.; Silverman, K. C.; Bills, G. F.; Pelaez, F.; Cascales, C.; Gibbs, J. B.; Lingham, R. B. *J. Org. Chem.* **1994**, *59*, 6296.

(8) (a) Singh, S. B.; Zink, D. L.; Bills, G. F.; Jenkins, R. G.; Silverman, K. C.; Lingham, R. B.*Tetrahedron Lett.* **1995**, *36*, 4935. (b) Singh, S. B.; Ball, R. G.; Bills, G. F.; Cascales, C.; Gibbs, J. B.; Goetz, M. A.; Hoogsteen, K.; Jenkins, R. G.; Lingham, R. B.; Silverman, K. C.; Zink, D. L. *J. Org. Chem.* **1996**, *61*, 7727.

(9) Jayasuriya, H.; Ball, R. G.; Zink, D. L.; Smith, J.; Goetz, M. A.; Jenkins, R. G.; Nallin-Omstead, M.; Silverman, K. C.; Bills, G. F.; Lingham, R. B.; Singh, S. B.; Pelaez, F.; Cascales, C. *J. Nat. Prod.* **1995**, *58*, 986.

(10) Singh, S. B.; Jones, E. T.; Goetz, M. A.; Bills, G. F.; Nallin-Omstead, M.; Jenkins, R. G.; Lingham, R. B.; Silverman, K. C.; Gibbs, J. B. *Tetrahedron Lett.* **1994**, *35*, 4693.

(11) (a) Jayasuriya, H.; Bills, G. F.; Cascales, C.; Zink, D. L.; Goetz, M. A.; Jenkins, R. G.; Silverman, K. C.; Lingham, R. B.; Singh, S. B. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2081. (b) Silverman, K. C.; Jayasuriya, H.; Cascales, C.; Vilella, D.; Bills, G. F.; Jenkins, R. G.; Singh, S. B.; Lingham, R. B. *Biochem. Biophys. Res. Commun.* **1997**, *232*, 478.

(12) (a) Omura, S.; Van Der Pyl, D.; Takahashi, Y.; Takeshima, H. *J. Antibiot.* **1993**, *46*, 222. (b) Shiomi, K.; Yang, H.; Inokoshi, J.; Van Der Pyl, D.; Nakagawa, A.; Takeshima, H.; Omura, S. *J. Antibiot.* **1993**, *46*, 229.

(13) Van Der Pyl, D.; Inokoshi, J.; Shiomi, K.; Yang, H.; Takeshima, H.; Omura, S. *J. Antibiot.* **1992**, *45*, 1802.

(14) Liu, W. C.; Barbacid, M.; Bulgar, M.; Clarck, J. M.; Crosswell, A. R.; Dean, L.; Doyle, T. W.; Fernandes, P. B.; Huang, S.; Manne, V.; Pirnik, D. M.; Wells, J. S.; Meyers, E. *J. Antibiot.* **1992**, *45*, 454.

(15) (a) Hara, M.; Akasaka, K.; Akinaga, S.; Okabe, M.; Nakano, H.; Gomez, R.; Wood, D.; Uh, M.; Tamanoi, F. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 2281. (b) Tamanoi, F. *Trend. Biochem. Sci.* **1993**, *18*, 349.

(16) (a) See refs 1f and 4d and references cited therein. (b) Omer, C. A.; Kral, A. M.; Diehl, R. E.; Prendergast, G. C.; Powers, S.; Allen, C. M.; Gibbs, J. B.; Kohl, N. E. *Biochemistry* **1993**, *32*, 5167. The IC_{50} of fusidienol A was determined by using filter binding assay essentially as described in ref 5d.

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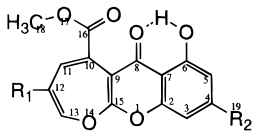

1a: R_1 = H, R_2 = CH_3 (Fusidienol A)
1b: R_1 = CH_2OH, R_2 = H (Fusidienol)

Omdel, Namibia, was grown for 17 days in liquid medium and extracted with methyl ethyl ketone. Gel permeation chromatography on Sephadex LH-20 followed by trituration of the fractions with methanol afforded 1.4 g/L fusidienol A (**1a**) that was recrystallized from acetone as prisms/cubes mp 202−203 °C.

Structure Elucidation

Electron impact (EI) mass spectral analysis of fusidienol A (**1a**) gave a molecular ion at *m/z* 300. High-resolution measurement of the molecular ion gave an empirical formula of $C_{16}H_{12}O_6$. This formula indicated that fusidienol A had 11 degrees of unsaturation and possessed one less oxygen atom compared to fusidienol (**1b**).[10] The UV spectrum of **1a** was also identical to that of fusidienol (**1b**) and gave absorption bands at 237 and 326 nm. Like fusidienol, **1a** showed absorption bands for hydroxy (3500 cm^{-1}), ester (1724 cm^{-1}), and highly conjugated carbonyl (1655 cm^{-1}) groups in the infrared spectrum. Comparison of ^{13}C NMR spectra (Table 1) of **1a** and **1b** indicated that these compounds were similar except for the absence of the signal for the hydroxy methyl carbon and the presence of an additional methyl carbon in the spectrum of **1a**. The APT spectrum of fusidienol A revealed a methyl carbon (δ 22.39), a methoxy carbon (δ 52.50), four olefinic/aromatic methines, and nine quaternary carbons. An overlapping carbon signal at δ 107.26 (assigned to a quaternary carbon and a methine by HMQC and HMBC, see below) was completely absent in the APT spectrum. This was due to the nulling of the opposite signs of the quaternary and methine carbons of a perfectly overlapped carbon signal in the APT spectrum of **1a**.

Examination of the ^1H NMR spectrum of **1a** in $CDCl_3$ (Table 1) indicated the presence of a methoxy group, a 1,2,3,5-tetrasubstituted aromatic ring, a monosubstituted diene, and an aromatic methyl group coupled to two *ortho* aromatic protons. These structural subunits were assembled into fusidienol A using HMBC correlations (Figure 1) using a $^nJ_{CH}$ = 7 Hz. The methoxy protons, as expected, gave correlations only to the ester carbonyl. Like fusidienol, two- and three-bond HMBC correlations of H-11 and H-13 to the respective carbons (see Figure 1) helped in assembling the oxepin ring in the left hand side of the molecule. The right hand side of the molecule was similarly assembled with the help of HMBC correlations of H-3,5 and H-19. The exchangeable proton at δ 12.05 gave correlations to C-5 (δ113.29), 6 (δ160.54), 7 (δ107.26). Unlike fusidienol, the chelated hydroxy proton did not show correlations to C-8 carbonyl. This may be due to the difference in the extent of intramolecular hydrogen bonding of the hydroxy proton to the carbonyl group affected by the solvent change (CD_3CN *vs* $CDCl_3$). The carbon signal at δ 107.26 gave HMQC correlations to the proton at δ 6.66 (H-3) and HMBC correlations to the chelated hydroxy proton (δ 12.05), hence confirming the assignment of δ 107.26 signal to both the quaternary carbon at C-7 and the methine carbon at C-3. The COSY correlations of the methyl group to the aromatic protons and respective HMBC correlations helped in the placement of the methyl group at C-4. Therefore, structure **1a** was proposed for fusidienol A. This structural proposal was supported by mass spectral fragmentation of **1a** (*vide infra*) and was finally confirmed by single-crystal X-ray analysis (Figure 2).[17]

Table 1. ^1H and ^{13}C NMR Assignments and HMBC Correlations of Fusidienol A in $CDCl_3$ at 400 MHz

position	δC	mult	δH	HMBC C → H
2	153.63	C°		H-3
3	107.26*	CH	6.66, dq, 1.2, 0.4	H-5, H-19
4	147.49	C°		H-19
5	113.29	CH	6.65, dq, 1.6, 0.8	H-3, 6-OH, H-19
6	160.54	C°		6-OH, H-5
7	107.26*	C°		H-3, H-5, 6-OH
8	182.26	C°		
9	104.77	C°		H-11, H-13 (weak)
10	130.80	C°		H-11
11	131.93	CH	6.94, d, 5.6	H-13
12	116.44	CH	5.87, t, 5.6	H-11 (weak), H-13 (weak)
13	146.71	CH	6.36, d, 5.6	H-11, H-12
15	161.72	C°		H-13
16	166.94	C°		H-11, H-18
18	52.50	CH_3	3.82, s	
19	22.39	CH_3	2.40, t, 0.4	H-3, H-5
6-OH			12.05, s	

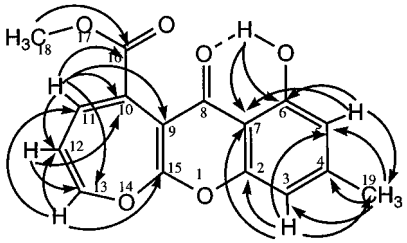

Figure 1. HMBC ($^nJ_{CH}$ = 7 Hz) correlations of fusidienol A.

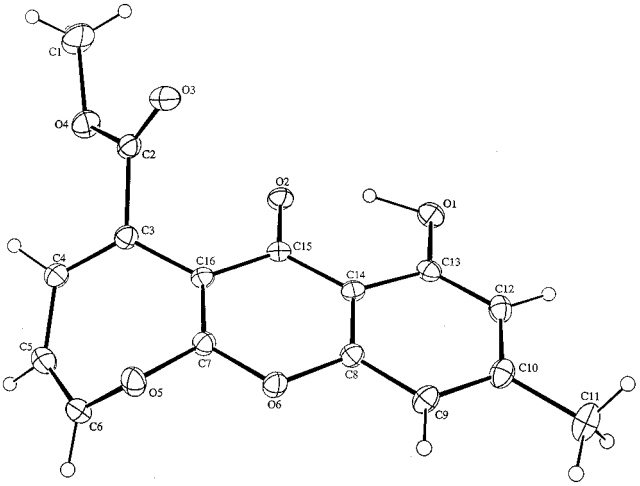

Figure 2. Perspective view of X-ray crystal structure of fusidienol A (**1a**).

Mass Spectral Fragmentation of Fusidienol A. EI mass spectral fragmentation of fusidienol A is outlined in Figure 3. The origin of the fragments were verified by MS/MS analysis. The fragmentation pattern seems to follow two distinct pathways. The first pathway appears to follow the aromatization route where the oxygen 14 in the oxepin ring is lost to give a xanthone-type fragment **2a** (top panel in Figure 3). The ion **2a** undergoes further fragmentation and loses a mole of

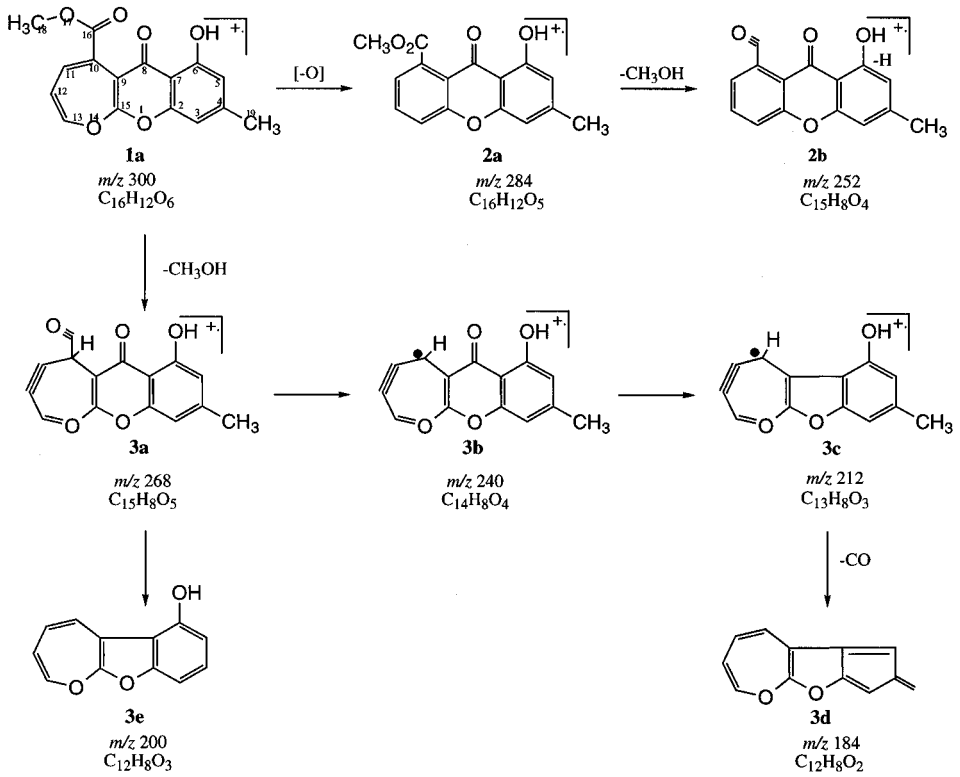

Figure 3. EI-MS/MS fragmentations of fusidienol A.

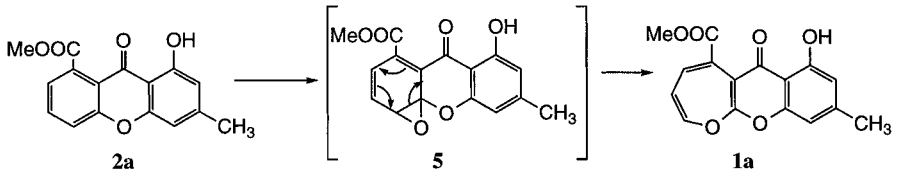

Figure 4. Proposed biogenesis of fusidienol A.

methanol to give fragment **2b** (*m*/*z* 252). No further fragmentation of this fragment ion was observed in MS/MS analysis. The second pathway initially retains the oxygen in the oxepin ring and undergoes two modes of fragmentation. In the first mode the fragment ion **3a** (*m*/*z* 268) undergoes a sequential loses of three molecules of CO to give fragment ions **3b** (*m*/*z* 240/241), **3c** (*m*/*z* 212), and **3d** (*m*/*z* 184), respectively. In the second mode, **3a** simultaneously loses 2 mol of CO and a methyl group to give the fragment ion **3e** (*m*/*z* 200).

Biological Activity

Fusidienol A exhibited an IC_{50} value of 1.8 μM against recombinant human FPTase and was not active against bovine brain GGTase at 50 μM (highest level tested). In cellular models of toxicity and Ras processing, fusidienol A was not toxic and did not inhibit Ras processing at 10 μM.[3a] These assays could not be performed at higher concentrations due to the insolubility of this compound in an aqueous system. The *in vitro* activity of fusidienol A (**1a**) is essentially identical to that of fusidienol (**1b**). Like fusidienol, this compound is also not competitive with either of the substrates.

Biogenesis

The most likely precursor of fusidienol A biogenesis may be a xanthone derivative of structure type **2a** (Figure 4). It is well-known[18] that the oxepin ring originates from benzene oxide and these two structural units exist in equilibrium. In this case epoxidation of **2a** would give epoxide **5**, which could rearrange to form fusidienol A (**1a**).

Experimental Section

General Experimental Procedures. For general experimental procedures, see refs 6 and 7.

Production of Fusidienol by *Phoma* Species. Stock cultures of MF 6118 were maintained as mixtures of spores and hyphae in sterile soil and stored at 4 °C until ready for use. The fungal isolate MF6118 is permanently maintained in the Merck

(17) **X-ray crystallography:** compound **1a**, $C_{16}H_{12}O_6$, $M_r = 300.270$, monoclinic, $P2_1/c$, $a = 8.132(3)$ Å, $b = 11.592(1)$ Å, $c = 14.633(1)$ Å, $\beta = 102.43(1)°$, $V = 1347.0(9)$ Å^3, $Z = 4$, $D_x = 1.481$ g cm^{-3}, monochromatized radiation λ(Cu Kα) = 1.541 838 Å, $\mu = 0.92$ mm^{-1}, $F(000) = 624$, $T = 294$ K. Data were collected on a Rigaku AFC5 diffractometer to a θ limit of 70°, which yielded 2770 unique reflections. The structure was solved by direct methods (SHELXS-86) and refined using full-matrix least-squares on F^2 (SHELXL-93). The final model was refined using 206 parameters and all 2770 data. All non-hydrogen atoms were refined with anisotropic thermal displacements. The final agreement statistics are as follows: $R = 0.045$ (based on 1911 reflections with $I \geq 3\sigma(I)$), $R_w = 0.115$, $S = 1.07$ with $(\Delta/\sigma)_{max} < 0.01$. The maximum peak height in a final difference Fourier map is 0.23(5) e Å^{-3}, and this peak is without chemical significance. The atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre. The coordinates can be obtained on request from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK.

(18) March, J. *Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*, 3rd ed.; Wiley: New York, 1985; pp 1026.

Microbial Resources Culture Collection. Seed cultures were inoculated by using a small portion of the preserved soil aseptically transferred into a 250 mL Erlenmeyer flask containing 50 mL of seed medium of the following composition (in g/L): corn steep liquor, 5.0; tomato paste, 40.0; oat flour, 10.0; glucose, 10.0; and trace elements solution, 10 mL/L (consisting of, in g/L: $FeSO_4 \cdot 7H_2O$, 1.0; $MnSO_4 \cdot 4H_2O$, 1.0; $CuCl_2 \cdot 2H_2O$, 0.025; $CaCl_2 \cdot 2H_2O$, 0.1; H_3BO_3, 0.056; $(NH_4)_6Mo7O_{24} \cdot 4H_2O$, 0.019; $ZnSO_4 \cdot 7H_2O$, 0.2; dissolved in 0.6 N HCl). The pH of the medium was adjusted to 6.8 by addition of NaOH before sterilization. Seed medium was prepared using distilled water and was dispensed into Erlenmeyer flasks that were capped with cotton plugs before being autoclaved at 121 °C for 20 min. Seed cultures were incubated at 25 °C, on a gyrotory shaker (220 rpm, 5.1 cm throw) for 72−73 h prior to inoculation of fermentation production flasks.

Production fermentations were performed in 250 mL Erlenmeyer flasks containing 44 mL of liquid production medium formulated as follows: corn meal, 50.0 g; sucrose, 80.0 g; yeast extract, 1.0 g; and distilled water to 1 L. Liquid medium production flasks were capped with cotton plugs and sterilized at 121 °C for 15 min. Each production flask was inoculated with 2.0 mL of vegetative seed growth. Production flasks were incubated one-third at 22 °C, one-third at 25 °C, and one-third at 27 °C on a gyrotory shaker (220 rpm, 5.1 cm throw) for 17 days.

Isolation of Fusidienol A. The fermentation broth (0.78 L) was extracted twice with 1 L each of methyl ethyl ketone by shaking the flasks for 2 h on a shaker. The extract was collected by filtration through a bed of Celite. Methyl ethyl ketone was removed under reduced pressure on a rotatory evaporator and finally lyophilized to give 4.5 g of solids. The solid material was triturated with 200 mL of methanol and filtered to give filtrate A and undissolved solids. The undissolved solid material was heated at 50 °C for 30 min in 1:1 ethyl acetate and acetone (200 mL) and filtered to give filtrate B and residue. Filtrate B was slowly concentrated under reduced pressure to give thick yellowish crystals of fusidienol A (**1a**), which were collected by filtration. A significant amount of fusidienol A (**1a**) was also present in filtrate A, which was purified by chromatography on a 2.0 L Sephadex LH-20 column. Elution of the column with methanol followed by trituration with methanol and filtration gave additional amounts of fusidienol A. The total yield of fusidienol A was 1.4 g/L. Fusidienol A was crystallized from acetone as cubes/prisms: mp 202−203 °C; UV (CH_3OH) λ_{max} 237 (ϵ = 19 068), 326 (6675) nm; IR (ZnSe) ν_{max} 3100, 2955, 1724, 1655, 1606, 1493, 1434, 1363, 1267, 1208, 1152, 1118, 1089, 1054, 1018, 964, 918, 885, 813, 791, 754, 715, 700, 666 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); HREIMS *m*/*z* 300.0652 (M^+, 93, calcd for $C_{16}H_{12}O_6$ 300.0634), 284.0685 (5, calcd for $C_{16}H_{12}O_5$ 284.0685), 268.0389 (100, calcd for $C_{15}H_8O_5$ 268.0372), 241.0501 (14, calcd for $C_{14}H_9O_4$ 241.0501), 240.0416 (8, calcd for $C_{14}H_8O_4$ 240.0423), 212.0459 (12, calcd for $C_{13}H_8O_3$ 212.0473), 200.0505 (5, calcd for $C_{12}H_8O_3$ 200.0473), 184.0523 (9, calcd for $C_{12}H_8O_2$ 184.0524), 150.0335 (6, calcd for $C_8H_6O_3$ 150.0317), 133.0303 (5, calcd for $C_8H_5O_2$ 133.0290), 128.0626 (7, calcd for $C_{10}H_8$ 128.0626).

Supporting Information Available: ^1H and ^{13}C NMR spectra and X-ray crystallographic data of **1a** (7 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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