PRODUCTION OF NEW POLYENE ANTIBIOTICS BY *STREPTOMYCES CELLULOSAE* AFTER ADDITION OF ETHYL (*Z*)-16-PHENYLHEXADEC-9-ENOATE

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Ethyl (*Z*)-16-phenylhexadec-9-enoate (**3**), an analog of ethyl oleate (**2**), was synthesized and added to cultures of *Streptomyces cellulosae* ATCC 12625 which normally produce fungichromin (**1**) as the principal polyene antibiotic. These cultures showed drastic reduction of fungichromin biosynthesis but afforded four new polyene antibiotics with a truncated four carbon side chain which are designated as isochainin (**11**) (an isomer of chainin (**10**)), 14-hydroxyisochainin (**12**), 1′-hydroxyisochainin (**13**), and 1′,14-dihydroxyisochainin (**14**). The close correspondence of ^{13}C NMR chemical shifts between these compounds and fungichromin suggests that the stereochemistry at every site is exactly analogous.

The medical importance of polyene antibiotics,[1] particularly as antifungal agents, continues to spur efforts aimed at their isolation, structure elucidation, and chemical synthesis.[2~7] One of these compounds, fungichromin (**1**), is normally the principal polyene produced by fermentations of *Streptomyces cellulosae* ATCC 12625.[1,8] Our recent biosynthetic studies show that fungichromin (**1**) is derived from one propionate unit, twelve acetate units, and one intact octanoate unit condensed in the fashion typical of polyketide biogenesis (Fig. 1).[9] Labeling experiments demonstrate that the biological source of the octanoate is exclusively oleate (acetate is not significantly incorporated into the octanoate unit of **1**).[9] This agrees with the observation that esters of oleic acid (*e.g.*, ethyl oleate (**2**)) must be added to the fermentation medium to obtain significant production of fungichromin (**1**).[10] Hence it seemed possible that addition of analogs of **2** like ethyl (*Z*)-16-phenylhexadec-9-enoate (**3**) could generate new polyene antibiotics bearing an altered side chain. The present report describes these experiments and the structures of resulting pentaene antibiotics.

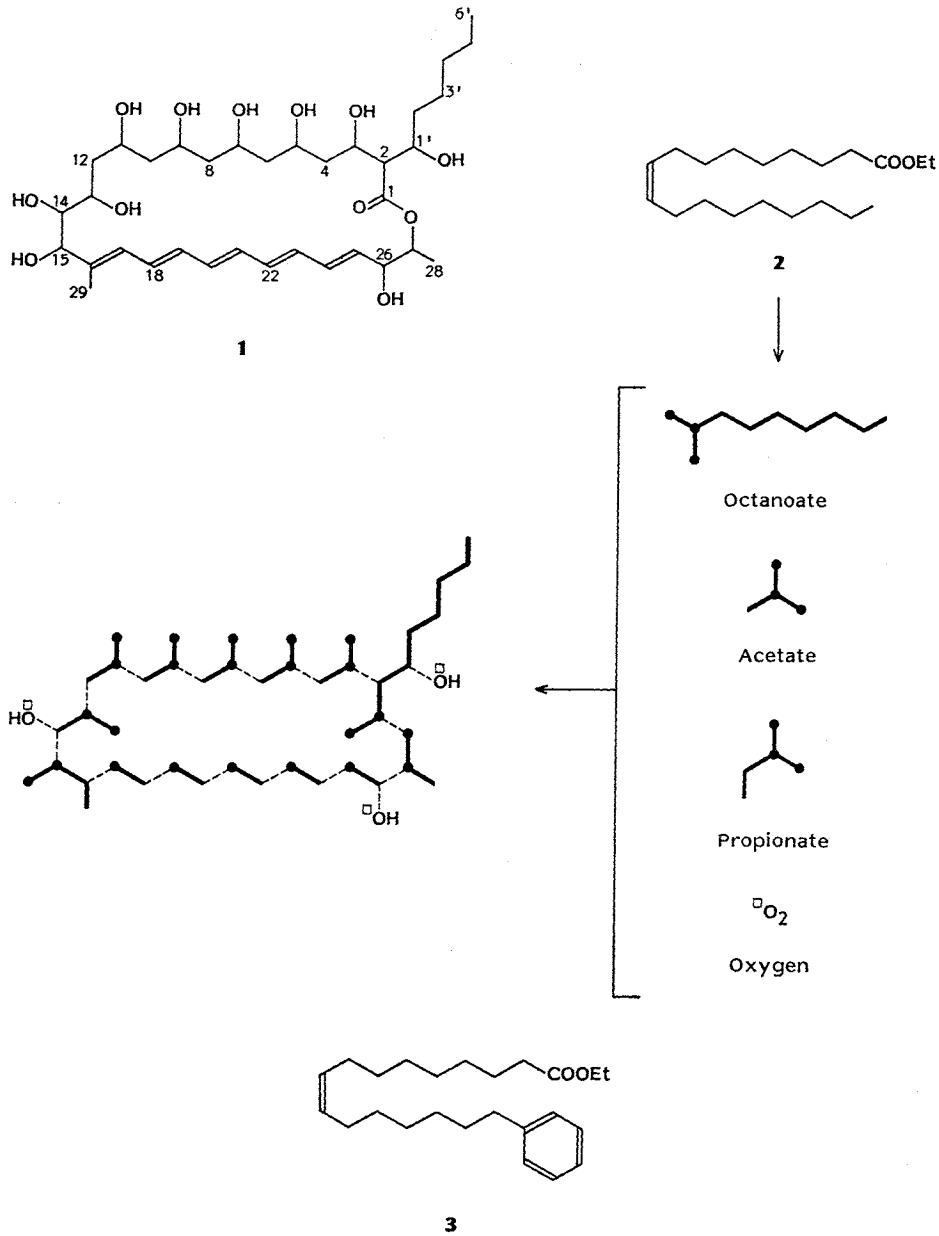
Experimental

General methods, instrumentation, and procedures for fermentation of *S. cellulosae* ATCC 12625 and isolation of fungichromin (**1**) have been described previously.[9] Commercial reagents were purchased from Aldrich or Sigma Chemical Company unless otherwise stated. IR spectra were obtained on a Nicolet 7199 FT-IR spectrometer. NMR spectra were measured in 5 mm tubes at 25°C on Bruker WH200, AM300, and AM400 instruments in the specified solvent with TMS as internal standard for ^1H NMR spectra and solvent peaks referenced to TMS as internal standard for ^{13}C NMR spectra. UV spectra were recorded on a Hewlett Packard 8450A Diode Array Spectrophotometer. MS were obtained on Kratos AEI MS-50 (high-resolution electron impact (HREI), 70 eV) and MS-9 (positive ion fast atom bombardment (FAB), Ar) instruments. HPLC employed a Hewlett Packard 1082B instrument with a variable UV detector set to 357 nm.

5-Phenylpentyl *p*-Toluenesulfonate (**4**)

A solution of 5-phenylpentanol (24.3 g, 148 mmol) in CH_2Cl_2 (200 ml) and pyridine (15.8 g, 200

Fig. 1. Biosynthetic origin of fungichromin (**1**) and structure of oleate analog **3**.



mmol) at 0°C was treated with *p*-toluenesulfonyl chloride (31.1 g, 163 mmol), and stirred 12 hours at 20°C. The solution was concentrated *in vacuo*, the residue was dissolved in hexane - EtOAc (600 ml, 9:1), and the resulting solution was cooled to −78°C and filtered to give **4** (41.7 g, 88%): IR ($CHCl_3$ cast) cm^{-1} 1598, 1359, 1177; ^1H NMR (400 MHz, $CDCl_3$) δ 1.46 (2H, m), 1.52~1.70 (4H, m), 2.43 (3H, s), 2.55 (2H, t, J=8 Hz), 4.03 (2H, t, J=6.8 Hz), 7.10~7.38 (7H, m), 7.80 (2H, m); MS *m/z* 318.1275 (M, $C_{15}H_{22}O_3S$), 146.1095 (M−$C_7H_8O_3S$), 104.0629 (C_8H_8), 91.0549 ($PhCH_2$).

Anal Calcd for $C_{15}H_{22}O_3S$: C 67.89, H 6.97, S 10.07.
Found: C 68.15, H 6.95, S 10.27.

7-Phenylheptanoic Acid (5)

A solution of sodium ethoxide in ethanol (made by adding sodium (3.31 g, 144 mmol) to ethanol (150 ml)) was added dropwise to a mixture of diethyl malonate (23.1 g, 144 mmol) and **4** (41.7 g, 131 mmol) kept at 80°C, and heating at 80°C was continued for 12 hours. The mixture was cooled and a solution of KOH (22.0 g, 392 mmol) in water (150 ml) was added. The solution was heated an additional 3 hours at 80°C. The cooled solution was acidified with 6 N HCl and extracted with ether (3×200 ml). The dried extracts (Na_2SO_4) were concentrated *in vacuo* and heated at 160°C for 3 hours. Distillation *in vacuo* (0.5 mmHg) at 147~152°C afforded the known[11] acid **5** (19.0 g, 70%): IR ($CHCl_3$ cast) cm^{-1} 3300~2500, 1708; ^1H NMR (400 MHz, $CDCl_3$) δ 1.30~1.45 (4H, m), 1.53~1.70 (4H, m), 2.32 (2H, t, J=7.4 Hz), 2.61 (2H, t, J=7.6 Hz), 7.12~7.32 (5H, m), 10.3 (1H, br); MS *m/z* 206.1308 (M, $C_{13}H_{18}O_2$), 104.0628 (C_8H_8), 91.0549 ($PhCH_2$).

7-Phenylheptanal (6)

Lithium aluminum hydride (5.0 g, 130 mmol) in THF (100 ml) was added dropwise to a solution of **5** (18.9 g, 92 mmol) in THF (50 ml) at 0°C over 1 hour. The solution was warmed to 20°C for 1 hour, methanol (30 ml) was added, and the mixture was poured into 1 N HCl (200 ml). This was filtered and extracted with ether (3×150 ml). The dried (Na_2SO_4) extracts were concentrated *in vacuo* and distilled (113~116°C, 0.35 mmHg) to give the known[12] 7-phenylheptanol (15.8 g, 89%): IR ($CHCl_3$ cast) cm^{-1} 3540, 1030; ^1H NMR (400 MHz, $CDCl_3$) δ 1.32 (6H, m), 1.44~1.65 (4H, m), 2.20 (1H, br s), 2.58 (2H, t, J=8 Hz), 3.57 (2H, t, J=6.8 Hz), 7.13 (3H, m), 7.25 (2H, m); MS *m/z* 192.1513 (M, $C_{13}H_{20}O$), 174.1410 (M−H_2O), 104.0629 (C_8H_8), 91.0549 ($PhCH_2$).

A solution of oxalyl chloride (20 ml, 230 mmol) in CH_2Cl_2 (250 ml) was treated with DMSO (34 ml, 440 mmol) at −60°C. The mixture was stirred 5 minutes at −60°C and a solution of 7-phenylheptanol (4.37 g, 22.7 mmol) in CH_2Cl_2 (20 ml) was added over 10 minutes. Stirring was continued for 15 minutes, triethylamine (70 ml, 0.5 mol) was added, and the mixture was allowed to warm to 20°C. This was washed with water (300 ml), 1 N HCl (300 ml), and 5% Na_2CO_3 (300 ml). The dried ($MgSO_4$) organic phase was concentrated *in vacuo* and distilled (130°C, 0.5 mmHg) to afford **6** (1.73 g, 40%): IR ($CHCl_3$ cast) cm^{-1} 1726, 1179; ^1H NMR (200 MHz, $CDCl_3$) δ 1.28~1.72 (8H, m), 2.41 (2H, dt, J=3 and 7 Hz), 2.60 (2H, t, J=8 Hz), 7.11~7.33 (5H, m), 9.77 (1H, t, J=3 Hz); MS *m/z* 190.1356 (M, $C_{13}H_{18}O$), 104.0632 (C_8H_8), 91.0549 ($PhCH_2$).

9-Bromononanoic Acid (7)

A solution of sodium *meta*-periodate (87.7 g, 410 mmol) in water (300 ml) was added to a solution of 9-bromononanol[13] (22.3 g, 100 mmol) in CH_3CN (200 ml) and CCl_4 (200 ml). $RuCl_3 \cdot 3H_2O$ (0.58 g, 2.2 mmol) was added, the mixture was stirred at 20°C for 2.5 hours, and the solution was extracted with CH_2Cl_2 (3×200 ml). The dried extracts (Na_2SO_4) were concentrated *in vacuo*, redissolved in ether (300 ml), filtered through a Celite 545 column (5×15 cm), and again concentrated *in vacuo*. The resulting solid was distilled (143°C, 0.25 mmHg) to afford the known[14] acid **7** (21.1 g, 89%): MP 36.0~36.3°C (literature[15] mp 36.0~36.5°C); IR ($CHCl_3$ cast) cm^{-1} 3300~2500, 1699; ^1H NMR (200 MHz, $CDCl_3$) δ 1.24~1.54 (8H, m), 1.54~1.75 (2H, m), 1.80~1.96 (2H, m), 2.35 (2H, t, J=8 Hz), 3.40 (2H, t, J=7 Hz), 8.94~9.14 (1H, br s); MS *m/z* 238.0450, 236.0416 (M, $C_9H_{17}BrO_2$), 157.1169 (M−HBr).

Ethyl 9-Bromononanoate (8)

A solution of **7** (21.0 g, 88.6 mmol) in ether (150 ml) was treated with thionyl chloride (12.7 g, 107 mmol) and heated to reflux for 4 hours. The cooled solution was concentrated *in vacuo*, redissolved in ether (150 ml), cooled to 0°C, and treated with excess ethanol (10 ml). The mixture was concentrated *in vacuo* and distilled (bp 113°C, 0.2 mmHg) to give the known[16] ester **8** (23 g, 98%): IR ($CHCl_3$ cast) cm^{-1} 2932, 1735, 1180; ^1H NMR (300 MHz, $CDCl_3$) δ 1.20~1.50 (8H, m), 1.26 (3H, t, J=7.2 Hz), 1.62 (2H, m), 1.85 (2H, m), 2.28 (2H, t, J=7.4 Hz), 3.41 (2H, t, J=6.9 Hz), 4.13 (2H, q, J=7.2 Hz); MS *m/z* 266.0709, 264.0730 (M, $C_{11}H_{21}BrO_2$), 185.1547 (M−HBr).

Ethyl 9-Iodononanoate (9)

A mixture of **8** (23.0 g, 86.7 mmol) and sodium iodide (15.6 g, 104 mmol) in 2-butanone (200 ml)

was heated to reflux with stirring for 18 hours. Water (200 ml) was added to the cooled mixture, and the solution was extracted with CH_2Cl_2 (3×100 ml). The dried extracts (Na_2SO_4) were concentrated *in vacuo* and distilled (131°C, 0.2 mmHg) to give **9** (26.5 g, 97%): IR ($CHCl_3$ cast) cm^{-1} 2929, 1735, 1177; ^1H NMR (200 MHz, $CDCl_3$) δ 1.20~1.50 (8H, m), 1.27 (3H, t, J=7.2 Hz), 1.62 (2H, m), 1.82 (2H, m), 2.29 (2H, t, J=7.4 Hz), 3.19 (2H, t, J=7.0 Hz), 4.12 (2H, q, J=7.2 Hz); MS m/z 312.0590 (M, $C_{11}H_{21}IO_2$), 185.1542 (M−HI).

Ethyl (*Z*)-16-Phenylhexadec-9-enoate (**3**)

Triphenylphosphine (3.30 g, 12.6 mmol) and **9** (3.60 g, 11.5 mmol) in toluene (20 ml) were heated to reflux for 12 hours. The mixture was cooled to 20°C, most of the toluene was removed by syringe, DMF (100 ml) was added, and the solution was cooled to −60°C. To this was added a solution of $LiN(SiMe_3)_2$ prepared by adding butyllithium (7 ml, 1.4 M in hexane) to hexamethyldisilazane (2.34 ml, 11 mmol) in THF (6 ml) at −78°C. The ylide solution was treated with aldehyde **6** (1.73 g, 9.1 mmol) in DMF (20 ml), stirred 1 hour at −60°C, and then warmed to 20°C. Acetic acid (1 N, 6 ml) was added followed by water (100 ml). The product was extracted into hexane (3×150 ml), dried (Na_2SO_4), and concentrated *in vacuo*. The residue was separated by column chromatography (Merck silica gel 60, 70~230 mesh) using hexane - EtOAc (98 : 2) to give **3** (1.65 g, 51%): IR ($CHCl_3$ cast) cm^{-1} 2930, 2855, 1737, 1653; ^1H NMR (400 MHz, $CDCl_3$) δ 1.23 (3H, t, J=7.1 Hz), ~1.3 (14H, m), 1.60 (4H, m), 2.00 (4H, m), 2.28 (2H, t, J=7.6 Hz), 2.60 (2H, t, J=7.8 Hz), 4.11 (2H, q, J=7.1 Hz), 5.34 (2H, t, J=4.6 Hz), 7.17 (3H, m), 7.26 (2H, m); MS m/z 358.2919 (M, $C_{24}H_{38}O_2$), 312.2453 (M−C_2H_5OH), 104.0626 (C_8H_8), 91.0549 ($PhCH_2$).

Anal Calcd for $C_{24}H_{38}O_2$: C 80.39, H 10.68.
Found: C 80.81, H 10.70.

Production and Isolation of **11**~**14**

Fermentation of *S. cellulosae* was done in the usual fashion[9] except that in the final culture (700 ml) **3** was added in varying amounts (0.5, 1.0, 2.0, and 5.0 g/liter) as a replacement for oleate esters (*e.g.* Span 85). After 7 days of fermentation the yellow-orange cultures were extracted and the polyene fraction was purified through the Sephadex LH-20 stage as before[9] to give 140 mg of pale yellow solid. This was dissolved in methanol (3 ml), water was added (2.7 ml), and the resulting precipitate was removed by centrifugation. HPLC separation (Waters C_{18} Radial Pak column, methanol - water (60 : 40), flow 1.00 ml/minute) of the supernatant afforded **14** (1.0 mg, retention time (t_R) 8.37 minutes), **13** (3.3 mg, t_R 10.43 minutes), **1** (2.1 mg, t_R 16.1 minutes), **12** (1.0 mg, t_R 16.1 minutes), and **11** (2.7 mg, t_R 25.8 minutes). To separate **1** and **12** which have identical retention times under these conditions, HPLC was repeated using methanol - water (50 : 50) (for **1**: t_R 34.7 minutes; for **12**: t_R 37.3 minutes).

Isochainin (**11**): MP ~190°C (dec); $[\alpha]_D^{25}$ −24.4° (c 0.16, MeOH); UV λ_{max} (THF - H_2O, 1 : 9) nm (ε) 307 (18,337), 321 (25,525), 338 (30,885), 357 (27,286); IR (MeOH cast) cm^{-1} 3350, 1723, 1700, 1046, 848; ^1H NMR (400 MHz, CD_3OD) δ 0.90 (3H, t, J=7.0 Hz), 1.2~1.5 (*ca.* 21H, m), 1.77 (3H, s), 2.31 (1H, ddd, J=11, 7 and 4 Hz), 3.30 (1H, m), 3.81 (1H, m), 3.91~4.04 (5H, m), 4.15 (1H, dd, J=10.4 and 4.4 Hz), 4.89 (1H, m), 5.92 (1H, dd, J=14.6 and 6.0 Hz), 6.06 (1H, d, J=11 Hz), 6.25~6.43 (6H, m), 6.51 (1H, dd, J=14.6 and 11 Hz); see Table 1 for ^{13}C NMR; positive ion FAB-MS (glycerol) m/z 633.56 (M·Na, $C_{33}H_{54}O_{10}$·Na), 610.45 (M, $C_{33}H_{54}O_{10}$).

14-Hydroxyisochainin (**12**): UV λ_{max} (THF - H_2O, 1 : 9) nm (ε) 307 (27,180), 322 (35,789), 339 (45,313), 358 (41,403); IR (MeOH cast) cm^{-1} 3260, 1720, 1705, 1046, 849; ^1H NMR (400 MHz, CD_3OD) δ 0.90 (3H, t, J=7.0 Hz), 1.2~1.6 (*ca.* 19H, m), 1.78 (3H, s), 2.34 (1H, ddd, J=11, 7 and 4 Hz), 3.25 (1H, m), 3.69~4.04 (8H, m), 4.83 (1H, m), 5.98 (1H, dd, J=14.8 and 5.2 Hz), 6.06 (1H, br d, J=11.4 Hz), 6.24~6.53 (7H, m); see Table 1 for ^{13}C NMR; positive ion FAB-MS (glycerol) m/z 649.55 (M·Na, $C_{33}H_{54}O_{11}$·Na), 626.50 (M, $C_{33}H_{54}O_{11}$).

1′-Hydroxyisochainin (**13**): UV λ_{max} (THF - H_2O, 1 : 9) nm (ε) 306 (12,197), 321 (16,360), 340 (17,045), 357 (15,682); IR (MeOH cast) cm^{-1} 3343, 1725, 1705, 845; ^1H NMR (400 MHz, CD_3OD) δ 0.92 (3H, t, J=7.0 Hz), 1.27~1.54 (*ca.* 19H, m), 1.78 (3H, s), 2.54 (1H, dd, J=7.6 and 7.2 Hz), 3.22 (1H, dt, J=10 and 2.5 Hz), 3.82~4.21 (6H, m), 4.07 (1H, br d, J=5.0 Hz), 4.12 (1H, dd, J=10.4 and 5.6 Hz), 4.88 (1H, m), 5.98 (1H, dd, J=14.6 and 5.0 Hz), 6.03 (1H, br d, J=11.6 Hz), 6.25~

Table 1. ^{13}C chemical shifts (δ) for fungichromin (**1**), isochainin (**11**), 14-hydroxyisochainin (**12**), 1′-hydroxyisochainin (**13**) and 1′,14-dihydroxyisochainin (**14**).

Carbon	1[a,b]	11[a]	12[a]	13[a]	14[a]
29	11.74	11.45	11.80	11.08	11.70
6′	14.38	—	—	—	—
28	17.96	18.29	18.30	17.95	17.91
5′	23.65	—	—	—	—
3′	26.01	23.60	23.61	19.51	19.52
4′	32.88	14.21	14.25	14.23	14.23
2′	36.22	29.87	30.18	38.36	38.40
12	39.58	42.52	39.50	41.58	39.54
4	41.38	42.70	42.33	42.86	41.34
10	44.34	44.20	44.15	44.18	44.36
6	45.17	44.91	44.83	45.17	45.21
8	45.33	45.11	45.16	45.26	45.36
2	60.35	54.26	54.40	60.31	60.46
13	70.34	67.50	70.26	67.47	70.38
11	71.45	71.00	71.35	71.12	71.46
1′	72.59	30.60	30.57	72.28	72.21
26	73.25	73.15	73.44	73.15	73.30
3	73.41	73.29	73.55	73.60	73.30
7	73.92	73.38	73.56	73.65	73.90
5	74.08	73.55	73.64	73.65	74.08
9	74.20	74.24	74.02	73.91	74.17
27	75.25	74.47	74.58	75.10	75.25
14	78.31	45.21	78.20	45.29	78.32
15	80.43	75.63	80.32	75.83	80.50
18	129.06	128.04	129.25	128.35	129.05
17	129.91	129.57	129.79	129.31	129.93
24	131.97	132.43	131.99	132.25	132.03
22	133.66	133.62	133.74	133.82	133.67
20	134.13	134.15	133.96	134.12	134.13
23	134.21	134.19	134.32	134.12	134.17
25	134.28	134.44	134.37	134.28	134.27
21	134.81	134.57	134.45	134.59	134.85
19	135.36	134.68	135.18	134.96	135.41
16	138.55	140.64	138.71	140.34	138.53
1	172.98	175.43	175.37	173.02	173.01

[a] 100.6 MHz ^{13}C NMR spectrum in methanol-d_4 with solvent reference at 49.00 ppm.

[b] For details of spectral assignment of fungichromin (**1**) see ref 9.

6.53 (7H, m); see Table 1 for ^{13}C NMR; positive ion FAB-MS (glycerol) *m/z* 649.51 (M·Na, $C_{33}H_{54}O_{11}$·Na), 626.50 (M, $C_{33}H_{54}O_{11}$).

1′,14-Dihydroxyisochainin (**14**): UV λ_{max} (THF - H_2O, 1 : 9) nm (ε) 306 (30,128), 322 (42,005), 340 (47,840), 358 (45,218); IR (MeOH cast) cm^{-1} 3335, 1723, 1705, 1049, 845; ^1H NMR (400 MHz, CD_3OD) δ 0.90 (3H, t, J=7.0 Hz), 1.11~1.70 (17H, m), 1.79 (3H, s), 2.56 (1H, dd, J=7.6 and 7.0 Hz), 3.27 (1H, br d, J=11 Hz), 3.71 (1H, dd, J=9 and 1.8 Hz), 3.82~4.22 (6H, m), 3.90 (1H, br d, J=9 Hz), 4.10 (1H, br d, J=4.6 Hz), 4.79 (1H, m), 6.01 (1H, dd, J=14.4 and 5.0 Hz), 6.06 (1H, br d, J=11.8 Hz), 6.22~6.51 (7H, m); see Table 1 for ^{13}C NMR; positive ion FAB-MS (glycerol) *m/z* 665.69 (M·Na, $C_{33}H_{54}O_{12}$·Na), 642.48 (M, $C_{33}H_{54}O_{12}$).

Preliminary Tests of Antifungal Activity of Compounds **11**~**14**

The disk diffusion method of BOYER[17] was used to compare the antifungal activity of **11**~**14**, and amphotericin B (Sigma Chemical Company). Five fungal strains obtained from Professor MICHAEL A. PICKARD (University of Alberta Microbiology Department), were examined: *Aspergillus*

terreus 327, *Cryptococcus ater* 164, *Mucor* sp., *Tolypocladium niveum* UAMH2742, and *Torulopsis utilis* var. *major* IMI33552. The surfaces of sterile agar plates (Difco potato - dextrose agar, 39 g/liter) were inoculated with suspensions of these organisms and paper disks containing concentrations of 40, 20, 10, and 3 μg/disk of antibiotic were placed on the surface. Plates were allowed to prediffuse at 4°C for 2 hours before incubation at 30°C. Diameters of inhibition zones were measured after 16, 24, and 32 hours. All compounds showed antifungal activity at 3 μg/disk against all organisms with the following exceptions: *C. ater* was resistant in this assay to all compounds tested including amphotericin B; compounds **13** and **14** did not inhibit *T. utilis* at up to 40 μg/disk; *A. terreus* was only inhibited at 20 μg/disk (or more) by **11** and **12** and at 40 μg/disk by **13**, **14**, and amphotericin B.

Results and Discussion

The oleate analog **3** was synthesized by the straightforward route depicted in Fig. 2. Commercially available 5-phenylpentanol was transformed to its tosylate **4** and extended by two carbon atoms *via* malonic ester synthesis to give 7-phenylheptanoic acid (**5**), which was converted to 7-phenylheptanal (**6**) by a reduction-reoxidation sequence (22% overall yield). The other half of **3** was prepared from 9-bromononanol[13] by oxidation to the corresponding acid **7**, esterification to **8**, and halogen exchange to produce ethyl 9-iodononanoate (**9**) in 85% overall yield. Wittig condensation of the triphenylphosphonium ylide derived from **9** with **6** gave a 51% yield of the desired *Z* isomer of ethyl 16-phenylhexadec-9-enoate (**3**). The stereochemistry of the double bond in **3** is known to be *cis* from the 10.5 Hz coupling constant between the olefinic hydrogens. Although these two protons

Fig. 2. Synthesis of **3**.

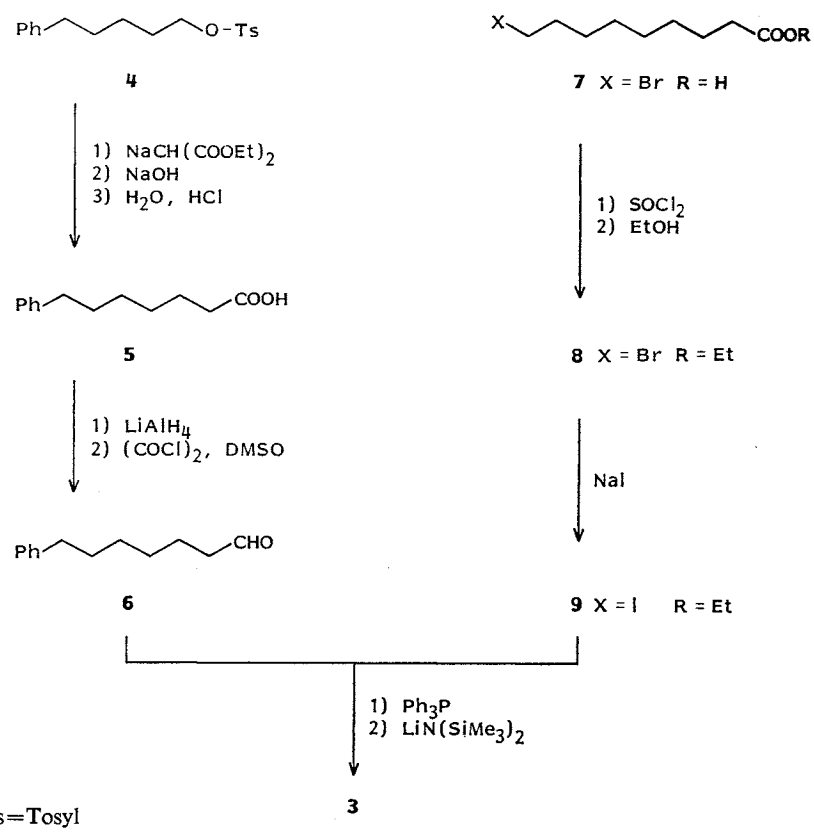
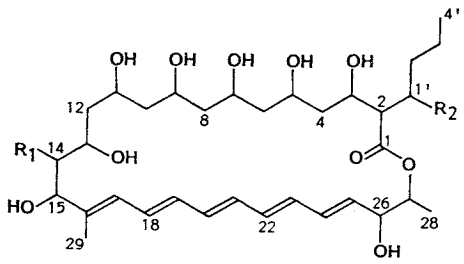
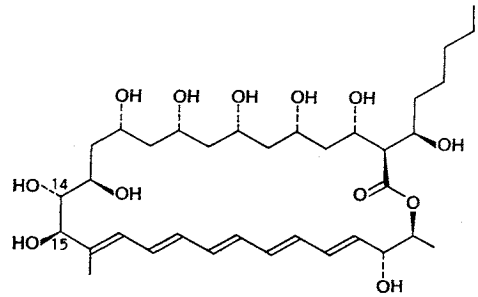


Fig. 3. Structures of chainin (**10**), new polyene macrolides (**11**~**14**), and pentamycin (**15**).



Chainin (**10**) R_1=H R_2=H
Isochainin (**11**) R_1=H R_2=H
12 R_1=OH R_2=H
13 R_1=H R_2=OH
14 R_1=OH R_2=OH



15a
15b (stereochemistry opposite at C-14 and C-15)

have nearly identical chemical shifts, the coupling can be seen in the ^{1}H NMR spectrum at the small satellite signals due to species bearing natural abundance carbon-13 at the olefinic carbons provided that the allylic hydrogens are simultaneously decoupled by homonuclear irradiation.

Compound **3** was added in varying amounts (0.5→5.0 g per liter) to growing cultures of *S. cellulosae* as a replacement for the oleate esters (*e.g.* Span 85 or **2**) normally used in the medium. Despite reasonably good growth of the organism, production of fungichromin (**1**) is greatly depressed by **3**. However, small quantities of four previously undetected polyene antibiotics could be isolated by HPLC in pure form. Our previous unambiguous assignment[9] of all ^{13}C NMR resonances of fungichromin (**1**) was the key tool for structure elucidation of these compounds. Comparison of the carbon chemical shifts (Table 1) shows very close correspondence except for two or three areas of structural difference. This information together with the positive ion FAB-MS and UV spectra characteristic of methylpentaenes (λ_{max} ~308, 324, 342, 358 nm) indicate that these polyenes are related to chainin (**10**).[1,18] Examination of IR and 60 MHz ^{1}H NMR spectra of chainin (**10**) (kindly provided by Professor K. L. RINEHART, Jr., University of Illinois) indicated that **11** possesses a very similar structure but could not conclusively distinguish between the two materials. Although an authentic sample of chainin (**10**) was not available, differences in optical rotation (for **11**: $[\alpha]_D^{25}$ −24.4° (*c* 0.16, MeOH); for **10**: $[\alpha]_D$ −112.2° (*c* 0.16 MeOH)) and decomposition point (for **11**: ~190°C; for **10**: 222~224°C) suggest that they may be stereoisomeric at one or more centers. We therefore designate compound **11** as isochainin. The other new polyenes are close relatives: 14-Hydroxyisochainin (**12**), 1′-hydroxyisochainin (**13**), and 1′,14-dihydroxyisochainin (**14**). All compounds showed antifungal activity roughly comparable to that of amphotericin B in preliminary tests.

The great similarity in ^{13}C NMR chemical shifts and the coproduction of compounds **11**~**14** and fungichromin (**1**) suggest that the stereochemistry at every site is exactly analogous. Recently the absolute stereochemistry of pentamycin, an antibiotic from *Streptomyces pentaticus* with the same gross structure as fungichromin (**1**), has been reported as being either **15a** or **15b**.[5] Elucidation of the stereochemical relationship between pentamycin (**15**) and fungichromin (**1**) should allow stereochemical assignment of isochainin (**11**) and its hydroxylated derivatives **12**~**14** with reasonable confidence.

The biochemical mechanism of action of **3** is presently unknown, but it may undergo partial β-oxidation to a truncated form which interferes with octanoate production or its attachment to the

growing polyketide chain. It is interesting that no polyenes bearing phenyl groups in the side chain could be detected. Further investigations on the effects of oleate analogs such as **3** and on incorporation of advanced biosynthetic precursors into polyene antibiotics are in progress.

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