Schizostatin, a Novel Squalene Synthase Inhibitor Produced by the Mushroom, *Schizophyllum commune*

II. Structure Elucidation and Total Synthesis

HIROSHI KOGEN*, KEIKO TAGO, SATORU KANEKO, KIYOSHI HAMANO†, KAORI ONODERA†, HIDEYUKI HARUYAMA††, KATSUHIRO MINAGAWA††, TAKESHI KINOSHITA††, TOMIO ISHIKAWA††, TATSUO TANIMOTO††† and YOSHIO TSUJITA†††

Exploratory Chemistry Research Laboratories,
†Biomedical Research Laboratories,
††Analytical and Metabolic Research Laboratories,
†††Pharmacology and Molecular Biology Research Laboratories, Sankyo Co., Ltd.,
1-2-58 Hiromachi, Shinagawa, Tokyo 140, Japan

(Received for publication February 29, 1996)

Schizostatin (**1**) has been isolated as a potent and selective inhibitor of squalene synthase. Its structure has been determined using spectroscopic methods: the compound is shown to be a diterpenoid which has a *trans*-dicarboxylic acid moiety. Total synthesis of schizostatin (**1**) was achieved by the highly regio- and stereoselective coupling reaction of an allylic bromide with a barium reagent. The *Z*-isomer **16** was also prepared using the stereoselective *syn*-addition of an organocopper reagent to acetylenedicarboxylate.

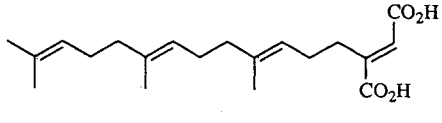
In our recent screening efforts for inhibitors of squalene synthase,[1] the novel inhibitor schizostatin (**1**)[2] was isolated as a fungal metabolite from the mushroom, *Schizophyllum commune* Fr. (SANK17785). The isolation, physico-chemical properties and biological activities of the compound, together with the taxonomy of the fungus, were reported in the preceding paper.[3] In this paper the structure elucidation and total synthesis are described. The synthesis of the *Z*-isomer **16** is also reported.

Results and Discussion

Structure Elucidation

The FAB-MS/MS (collisional activated dissociation, CAD) spectrum of $(M-H)^-$ ions at *m/z* 333 suggested that schizostatin might have two CO_2H groups (Fig. 2a). Moreover, Fig. 2a indicates the positions of double bonds in **1**. Furthermore, the positive-ion FAB-MS of **1** in the matrix of 3-nitrobenzylalcohol (3-NBA) saturated with LiI produced an abundant base peak of $(M-H+2Li)^+$

Fig. 1.



Schizostatin (**1**)

Fig. 2a. MS/MS fragmentation pattern of $(M-H)^-$ ions (*m/z* 333) in the negative-ion FAB-MS spectrum.

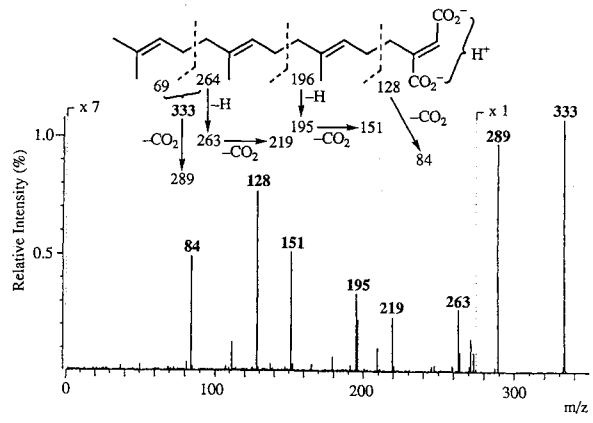
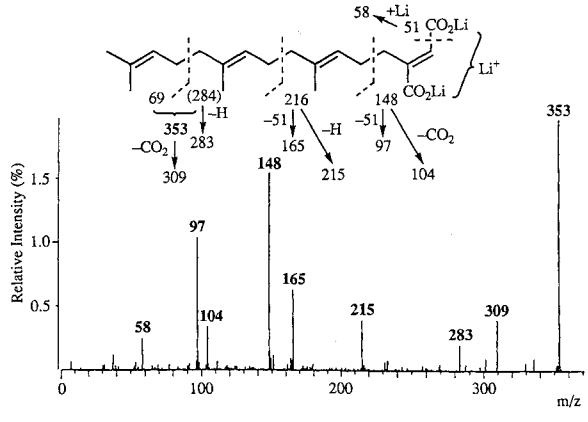


Fig. 2b. MS/MS fragmentation pattern of $(M-2H+3Li)^+$ ions (*m/z* 353) in the positive-ion FAB-MS spectrum.



ions. There were also relatively less abundant ions, $(M+Li)^+$ (70% relative abundance) and $(M-2H+3Li)^+$ ions (25% relative abundance). Generally, the FAB-MS of mono fatty acids in the matrix of 3-NBA saturated with LiI produce an abundant base peak of $(M+Li)^+$ ions.[4] The facts above indicated that **1** has two CO_2H groups. The structural information on the double bonds was also obtained from the CAD mass spectrum of $(M-2H+3Li)^+$ at *m/z* 353 (Fig. 2b). The ions at *m/z* 263, 195 and 128 in Fig. 2a, and the ions at *m/z* 283, 215 and 148 in Fig. 2b are the diagnostic ions for the assignment of the double bond positions. These ions result from the allylic bond cleavages, the so-called charge-remote fragmentations.[5~9]

The ^{13}C and ^1H NMR spectral data of **1** are summarized as shown in Table 1. The complete decoupled ^{13}C NMR spectrum in $CDCl_3$ gave 20 resolved peaks which were consistent with its molecular formula of $C_{20}H_{30}O_4$.[3] Since the twenty carbon signals were classified into $CH_3\times4$, $CH_2\times6$, $=CH\times4$ and six quaternary carbons by the DEPT spectrum, the sum of non-labile protons amounted to 28. Thus, the presence of two hydroxy groups were expected. The fact that the six quaternary carbons were further divided into four olefinic carbons and two oxy-carbonyl carbons from their chemical shifts suggested the presence of two carboxylic acid moieties.

The severe overlap of the signals in ^1H NMR spectrum, for example, three methyl groups around 1.6 ppm and olefinic protons at 5.09 ppm, made tracing of ^1H-^1H spin systems difficult. Thus, the intensive analysis of ^1H-^{13}C long range coupling by the HMBC spectrum was employed. The relatively better dispersion of ^{13}C signals compared to ^1H signals enabled to identification of three isoprene units and another modified isoprene unit with two carboxylic moieties as summarized in Fig. 3.

The stereochemistry at C-6 and C-10 was tentatively assigned as *E* from high field shifts of methyl carbons at C-7 and C-11 due to steric effects as commonly seen in this type of terpene. The stereochemistry at C-2 was determined as *E* by a $^{13}C\{^1H\}$NOE experiment wherein the enhancement of C-17 was observed along with C-1 and C-3 on irradiating H-2 (Fig. 4). Further confirmation of this was found in the actual synthesis of schizostatin (**1**).

Total Synthesis

A highly stereocontrolled total synthesis of schizostatin (**1**) was accomplished by following the work of COREY[10,11] and YAMAMOTO[12,13] in which establishing the stereochemistry of the trisubstituted dicarboxylic acid

Table 1. ^{13}C (125 MHz) and ^1H NMR (500 MHz) chemical shifts of schizostatin (**1**).

Carbon No.	^{13}C [a]	multiplicity [b]	^1H [c]	pattern [d]	*J* (Hz)
1	171.0	s			
2	127.6	d	6.90	s	
3	149.1	s			
4	27.9	t	2.86	t	4, 5 = 7.5
5	27.6	t	2.23	q	5, 6 = 7.5
6	122.5	d	5.19	t	
7	137.0	s			
7-Me	15.9	q	1.61	s	
8	39.7	t	1.98	q	8, 9 = 7.5
9	26.6	t	2.06	q	9, 10 = 7.5
10	124.1	d	5.10	t	
11	135.1	s			
11-Me	16.0	q	1.59	s	
12	39.7	t	1.97	q	12, 13 = 7.5
13	26.8	t	2.05	q	13, 14 = 7.5
14	124.4	d	5.09	t	
15	131.2	s			
15-Me	17.7	q	1.59	s	
16	25.7	q	2.00	s	
17	172.3	s			

[a] Sample was dissolved in $CDCl_3$. Chemical shifts shown with reference to $CDCl_3$ as 77.0 ppm.
[b] multiplicity s: quaternary d: methine t: methylene q: methyl.
[b] Chemical shifts shown with reference to TMS as 0.0 ppm.
[d] pattern s: singlet d: doublet t: triplet q: qualtet.

Fig. 3. ^{13}C-^1H long range couplings.

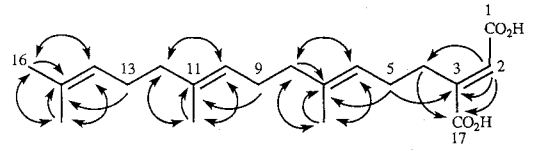
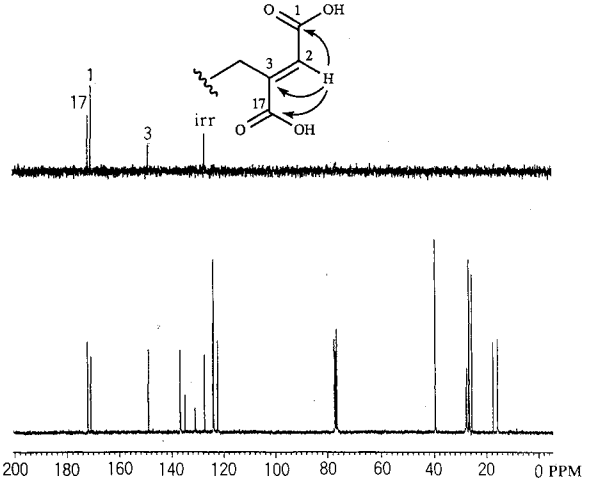


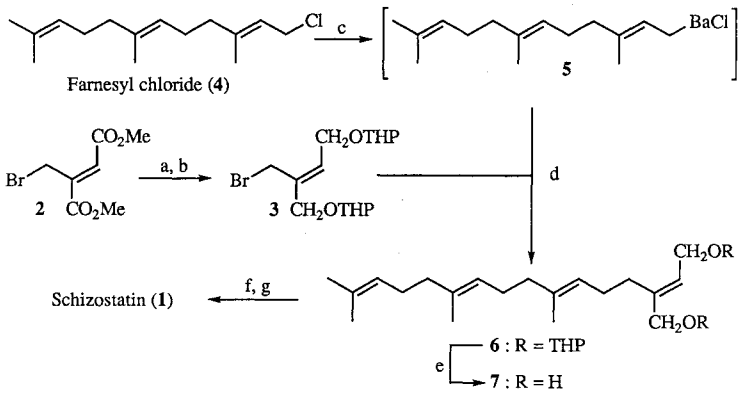
Fig. 4. $^{13}C\{^1H\}$ NOE differential spectra on irradiating H-2.



moiety was attainable by employing an organobarium reagent[14~16] as shown in Scheme 1. Thus commercially available farnesyl chloride (**4**) was treated with two equivalents of lithium napthalenide and barium iodide in THF at room temperature to give the organobarium reagent **5**; the subsequent reaction with the allyl bromide **3** which was prepared by diisobutylaluminum hydride reduction of **2**[17] gave conveniently the coupling product **6** in 71% yield as a single regio- and stereoisomer. Deprotection of compound **6** with a catalytic amount of pyridinium *p*-toluenesulfonate (PPTS) in methanol at room temperature provided the diol **7** in 88% yield. Oxidation of **7** in a sequential fashion using Swern and $NaClO_2$ conditions afforded schizostatin (**1**) in 72% yield after purification by reverse-phase Lobar column chromatography. Synthetic **1** was spectroscopically identical in all respects to natural schizostatin sample (HPLC, ^1H and ^{13}C NMR, IR, MS, and melting point).

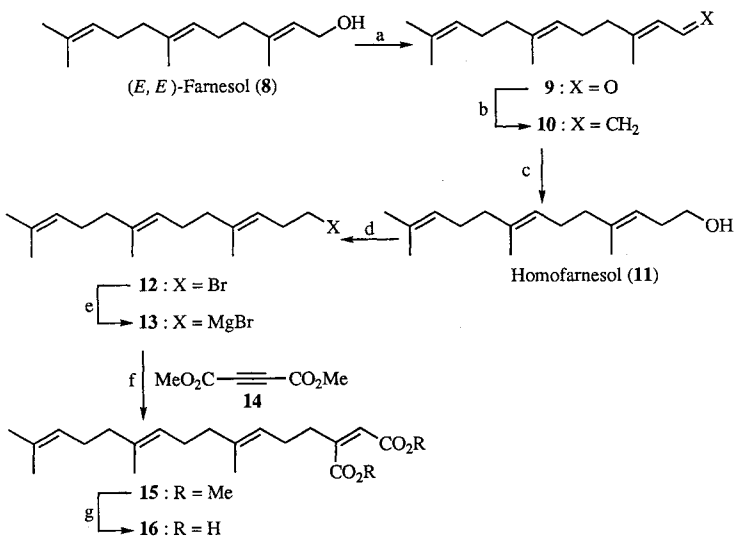
Synthesis of the *Z*-isomer **16** was initiated in order to evaluate its biological activity. Due to the instability of the *cis*-isomer of the allylic bromide **3**, a different strategy featuring stereoselective *syn*-addition of an organocopper reagent[18] to dimethyl acetylenedicarboxylate (**14**) was applied as means to synthesize the *Z*-isomer **16** as shown in Scheme 2. The homofarnesyl unit was prepared from (*E*, *E*)-farnesol (**8**) in an analogous procedure[19] to that described by LEOPOLD[20] for the synthesis of homogeraniol from geraniol. (*E*, *E*)-Farnesol (**8**) was trans-

Scheme 1.



a) 4.4 equiv of $(i\text{-Bu})_2AlH$, CH_2Cl_2, −78°C; b) DHP, cat. PPTS, CH_2Cl_2, r.t.; c) BaI_2, 2 equiv of lithium biphenylide, THF, r.t.; d) **3**, THF, −78°C; e) cat. PPTS, MeOH, r.t.; f) 2.6 equiv of $(COCl)_2$, Me_2SO, Et_3N, CH_2Cl_2, −78°C to r.t.; g) 2.5 equiv of $NaClO_2$, 20 equiv of NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH, H_2O, 0°C.

Scheme 2.



a) 1.2 equiv of $(COCl)_2$, Me_2SO, Et_3N, CH_2Cl_2, −60°C to r.t.; b) 1.1 equiv of Ph_3PCH_3Br, 1.1 equiv of NaHMDS, THF, r.t.; c) (i) 1.1 equiv of $(Sia)_2BH$, THF, 0°C to r.t.; (ii) 30% H_2O_2-3-N NaOH, r.t.; d) 1.1 equiv of Ph_3P, 1.1 equiv of Br_2, 2.0 equiv of pyridine, CH_2Cl_2, 0°C; e) 3.6 equiv of Mg, 1.1 equiv of $BrCH_2CH_2Br$, Et_2O, r.t.; f) (i) $CuBr\cdot Me_2S$, THF, −40°C; (ii) **14**, THF, −78°C; g) 10 equiv of LiOH, THF-H_2O, 40°C.

formed to farnesal (**9**) *via* Swern oxidation. The aldehyde **9** was treated with the Wittig reagent derived from methyltriphenylphosphonium bromide and sodium hexamethyldisilazane (NaHMDS) to give the tetraene **10** in 99% yield from **8**. Hydroboration of **10** with disiamylborane followed by oxidative treatment produced homofarnesol (**11**) in 93% yield. Homofarnesol (**11**) was treated with triphenylphosphine and bromine in the presence of pyridine to give homofarnesyl bromide (**12**)[19] in 97% yield. The bromide **12** was converted to the Grignard reagent **13** through treatment with activated magnesium in the presence of 1,2-dibromoethane[21] in anhydrous Et_2O at room temperature. The alkyl copper-dimethyl sulfide complex, generated from the Grignard reagent **13** with cuprous bromide-dimethyl sulfide complex in anhydrous THF at $-40°C$, reacted with dimethyl acetylenedicarboxylate (**14**) at $-78°C$ to obtain selectively the *Z*-dicarboxylate **15** in 76% yield. The dicarboxylate **15** was saponified with LiOH to give the *Z*-isomer of schizostatin **16** in 97% yield. Compound **16** was found to be 15-fold less potent as a competitive inhibitor of squalene synthase than schizostatin.[3]

Experimental

General

The melting points (mp) and boiling points (bp) are uncorrected. All reagents were commercially obtained except where noted. Where appropriate, reagents were purified prior to use. All nonaqueous reactions were performed using flame dried glassware under an atmosphere of dry nitrogen. Air- and moisture-sensitive liquid and solutions were transferred *via* syringe or stainless steel cannula. Tetrahydrofuran (THF) was distilled from sodium-benzophenone ketyl under a nitrogen atmosphere. Dichloromethane was distilled from calcium hydride under a nitrogen atmosphere. Dry diethylether (Et_2O) was used as purchased from Aldrich (anhydrous, 99.8%). Dimethyl sulfoxide (DMSO), pyridine, and triethylamine (Et_3N) were dried by storage over 4Å molecular sieves. Flash column chromatography was performed on Merck Art 9385 silica gel 60 (230~400 mesh).

Proton NMR (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a JEOL JNM-GX-400 spectrometer, ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectra on a JNM-GSX-500, ^{13}C (67.5 MHz) NMR spectrum on a JNM-GX-270. The chemical shifts of ^1H NMR spectra in $CDCl_3$ were given using tetramethylsilane as internal standard, while ^{13}C NMR used the solvent signal as 77.0 ppm. Low and high resolution mass spectra were obtained using a JEOL JMS-AX-505H spectrometer. FAB-MS and FAB-MS-MS (CAD) spectra were obtained on a JEOL JMS-SX/SX102A four-sector tandem mass spectrometer (BEBE configuration) operating at 10 kV acceleration potential. Parent ions were produced by bombardment with a beam of Xe atoms (6 keV), and focused into the collision cell in the third field-free region between MS-1 and MS-2 using a first-stage mass spectrometer (MS-1) at a mass resolving power of approximately 1500 (10% valley). Collisionally activated dissociation (CAD) was accomplished *via* the introduction of argon into the collision cell, which was floated at 5 kV, at a pressure sufficient to give approximately 80% attenuation of the parent ion beam.

(2*Z*)-2-Bromomethyl-2-[(1,4-bis(tetrahydro-2-pyranyloxy)]-butene (3)

To a solution of 2-bromomethyl-2-butene-1, 4-dioic acid dimethyl ester (**2**)[17] (3.24 g, 13.7 mmol) in CH_2Cl_2 (60 ml) was added diisobutylaluminum hydride (1.0 M-*n*-hexane solution, 60 ml, 60 mmol) at $-78°C$. After 45 minutes at this temperature, MeOH (6 ml) and water (6 ml) were added dropwise. The mixture was allowed to warm to room temperature and stirred for 30 minutes. The resulting mixture was filtered, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure to give a dialcohol as an air-sensitive colorless prisms (2.09 g). The product was used without further purification: mp 77~78°C (recrystallized from *n*-hexane-CH_2Cl_2); IR ν_{max} ($CHCl_3$) cm^{-1} 3275, 1200, 1015, 995; ^1H NMR (400 MHz, $CDCl_3$): δ 4.09 (2H, s), 4.26 (2H, s), 4.32 (2H, d, J=6.5 Hz), 5.88 (1H, tr, J=6.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.2, 58.6, 64.7, 130.1, 138.2; MS *m/z* 180 (M^+).

Anal Calcd for $C_5H_9BrO_2$: C 33.17, H 5.01.
Found: C 33.26, H 4.94.

To a solution of the unpurified alcohol (2.09 g, 11.5 mmol) in CH_2Cl_2 (50 ml) at room temperature was added 3,4-dihydro-2*H*-pyran (2.5 ml, 27.4 mmol) and a catalytic amount of pyridinium *p*-toluenesulfonate (PPTS). The reaction mixture was stirred for 24 hours, then poured into water and extracted with EtOAc. The organic layer was washed with water and brine successively, and dried over anhydrous $MgSO_4$. After evaporation, the residue was chromatographed on silica gel (*n*-hexane-EtOAc, 10:1), to give an allyl bromide **3** as a colorless oil (2.66 g, 56% from **2**): IR ν_{max} ($CHCl_3$) cm^{-1} 2950, 1130, 1120, 1025; ^1H NMR (400 MHz, $CDCl_3$) δ 1.53~1.87 (12H, m), 3.51~3.56 (2H, m), 3.85~3.91 (2H, m), 4.10 (2H, s), 4.04~4.19 (2H, m), 4.31~4.38 (2H, m), 4.66 (2H, tr, J=3.5 Hz), 5.86 (1H, tr, J=6.0 Hz); MS *m/z* 269 (M^+-Br), 247 ($M^+-C_5H_9O_2$).

(2*E*,6*E*,10*E*)-1-(Tetrahydro-2-pyranyloxy)-3-[(tetrahydro-2-pyranyloxy)methyl]-7,11,15-trimethyl-2,6,10,14-hexadecatetraene (6)

To a suspension of anhydrous BaI_2[13] (10.75 g, 27.5 mmol) in THF (120 ml) was added at room temperature, a solution of lithium biphenylide, which was prepared from freshly cut lithium (385 mg, 55 mmol) and biphenyl

(8.47 g, 55 mmol) in THF (140 ml), through a cannula under an argon stream. The reaction mixture was stirred for 30 minutes at room temperature. To the resulting dark brown suspension of reactive barium in THF was slowly added a solution of (*E*,*E*)-farnesyl chloride (**4**) (5.00 g, 20.7 mmol) in THF (15 ml) at −78°C. After being stirred for 30 minutes at this temperature, allyl bromide **3** (3.49 g, 10 mmol) was added to the reaction mixture. After 1.5 hours at the same temperature, saturated NH_4Cl aqueous solution (20 ml) was added to the reaction mixture, then extracted with EtOAc. The organic layer was washed with water, dilute sodium thiosulfate solution and brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. Purification of the crude product by chromatography on silica gel (*n*-hexane-EtOAc, 20:1) provided 3.35 g of **6** (71%) as a colorless oil: IR ν_{max} ($CHCl_3$) cm^{-1} 2945, 2855, 1120, 1020; ^1H NMR (400 MHz, $CDCl_3$) δ 1.53~2.17 (24H, m), 3.47~3.54 (2H, m), 3.84~3.91 (2H, m), 3.91 (1H, d, J=12.5 Hz), 4.09 (1H, dd, J=6.5, 12.5 Hz), 4.21 (1H, d, J=12.5 Hz), 4.31 (1H, dd, J=6.5, 12.5 Hz), 4.57~4.67 (2H, m), 5.08~5.15 (3H, m), 5.67 (1H, tr, J=6.5 Hz); MS *m/z* 474 (M^+).

(2*E*,6*E*,10*E*)-3-Hydroxymethyl-7,11,15-trimethyl-2,6,10,14-hexadecatetraen-1-ol (**7**)

To a solution of **6** (3.35 g, 7.05 mmol) in MeOH (30 ml) was added a catalytic amount of pyridinium *p*-toluenesulfonate (PPTS). The solution was stirred at room temperature for 48 hours, then concentrated, poured into water, and extracted with EtOAc. The organic layer was washed with water and brine, dried over anhydrous $MgSO_4$, and concentrated. Purification of the crude product by chromatography on silica gel (*n*-hexane-EtOAc, 1:1), furnished **7** as a colorless oil (1.90 g, 88%): IR ν_{max} ($CHCl_3$) cm^{-1} 3615, 2930, 1450, 1385; ^1H NMR (400 MHz, $CDCl_3$) δ 1.60 (9H, s), 1.68 (3H, s) 1.95~2.19 (12H, m), 4.10 (2H, d, J=5.5 Hz), 4.22 (2H, tr, J=5.5 Hz), 5.08~5.14 (3H, m), 5.70 (1H, tr, J=7.0 Hz); MS *m/z* 306 (M^+).

(1*E*,5*E*,9*E*)-6,10,14-Trimethyl-1,5,9,13-pentadecatetraene-1,2-dicarboxylic Acid (Schizostatin) (**1**)

DMSO (0.29 ml, 4.2 mmol) was added dropwise to a solution of oxalyl chloride (0.27 ml, 3.2 mmol) in CH_2Cl_2 (4 ml) at −70°C. Following gas evolution, the mixture was stirred for 10 minutes before a solution of dialcohol **7** (238 mg, 0.78 mmol) in CH_2Cl_2 (4 ml) was added dropwise. The resulting white suspension was stirred at −50°C for an additional 1 hour. Triethylamine (0.78 ml, 5.6 mmol) was then added dropwise, causing the solution to clear. The solution was warmed to 0°C then stirred for 20 minutes. The reaction was quenched at 0°C with saturated NH_4Cl aqueous solution (5 ml). The product was extracted with EtOAc, washed with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to give dialdehyde as a pale yellow oil (231 mg, 98%). The product was used without further purification: IR ν_{max} ($CHCl_3$) cm^{-1} 1700, 1685; ^1H NMR (400 MHz, $CDCl_3$) δ 1.52 (3H, s), 1.59 (3H, s), 1.60 (3H, s), 1.68 (3H, s) 1.95~2.07 (8H, m), 2.21 (2H, q, J=7.5 Hz), 2.73 (2H, tr, J=7.5 Hz), 5.06~5.12 (3H, m), 6.51 (1H, d, J=7.5 Hz), 9.67 (1H, s), 10.21 (1H, d, J=7.5 Hz); MS *m/z* 302 (M^+).

To the solution of the unpurified dialdehyde and 2-methyl-2-butene (8.5 ml) in *t*-BuOH (16 ml) was added a solution of sodium dihydrogen phosphate dihydrate (2.50 g, 16 mmol) in water (4 ml). A solution of sodium chlorite (80% purity) (216 mg, 1.91 mmol) in water (4 ml) was added dropwise to the resulting mixture at 0°C over 3 minutes. The reaction mixture was stirred at 0°C for 1.5 hours, diluted with Et_2O (50 ml). The ethereal layer was collected, and the aqueous layer was extracted with Et_2O. The combined organic extracts were washed with water and brine, dried over anhydrous $MgSO_4$. Evaporation of the Et_2O under reduced pressure yielded a white solid which was purified by column chromatography on RP-18 (Lobar Fertigsaule Große B LiChroprep RP-18, acetonitrile-water, 65:35), to give the schizostatin (**1**) as a colorless prisms (186 mg, 73% from **7**): mp 119~122°C; IR ν_{max} ($CHCl_3$) cm^{-1} 1705, 1695, 1410, 1265; ^1H NMR (400 MHz, $CDCl_3$) δ 1.60 (6H, s), 1.61 (3H, s), 1.68 (3H, s), 1.95~2.10 (8H, m), 2.23 (2H, q, J=7.5 Hz), 2.86 (2H, tr, J=7.5 Hz), 5.07~5.14 (2H, m), 5.18 (1H, tr, J=7.0 Hz), 6.89 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.9, 16.0, 17.7, 25.7, 26.6, 26.8, 27.6, 27.9, 39.7, 122.5, 124.1, 124.4, 127.6, 131.3, 135.1, 137.0, 149.1, 171.0, 172.2; MS *m/z* 334 (M^+).

Anal Calcd for $C_{20}H_{31}O_4$: C 71.82, H 9.04.
Found: C 71.74, H 9.01.

(2*E*,6*E*)-3,7,11-Trimethyl-2,6,10-dodecatrien-1-al (Farnesal) (**9**)

To a solution of oxalyl chloride (4.5 ml, 52 mmol) in CH_2Cl_2 (100 ml) was added DMSO (7.5 ml, 106 mmol) in CH_2Cl_2 (50 ml) at −60°C under nitrogen. After 18 minutes, (*E*,*E*)-farnesol (**8**) (10.00 g, 45 mmol) in CH_2Cl_2 (50 ml) was added to the solution. The resulting mixture was warmed to −50°C, and stirred for an additional 1 hour. To the reaction mixture was added triethylamine (25 ml, 180 mmol), then stirred for 20 minutes at 0°C. The reaction was quenched with water (160 ml) at 0°C. The organic layer was collected, and the aqueous phase was extracted with CH_2Cl_2. The combined extracts were washed with brine, 1% HCl, water, 5% Na_2CO_3, water, and brine successively, dried over anhydrous $MgSO_4$, and concentrated *in vacuo* to afford **9** (9.90 g, 100%). The crude oil was distilled under a reduced pressure to give the pure (*E*,*E*)-farnesal (**9**) as a faint yellow oil (6.76 g): bp 122~124°C (3 mmHg); IR ν_{max} ($CHCl_3$) cm^{-1} 2920, 1665; ^1H NMR (400 MHz, $CDCl_3$) δ 1.60 (3H, s), 1.61 (3H, s), 1.68 (3H, s), 1.96~2.09 (4H, m), 2.17 (3H, d, J=1.2 Hz), 2.19~2.27 (4H, m), 5.06~5.14 (2H, m), 5.89 (1H, dd, J=1.2, 8.0 Hz), 10.00 (1H, d, J=8.0 Hz); MS *m/z* 220 (M^+).

(3*E*,7*E*)-4,8,12-Trimethyl-1,3,7,11-tridecatetraene (**10**)

To a suspension of methyltriphenylphosphonium bromide (9.80 g, 27.5 mmol) in THF (100 ml) was added sodium bis(trimethylsilyl)amide (NaHMDS) (1.0 M-THF solution, 26.3 ml, 26.3 mmol) at −78°C, then warmed to 0°C. After reaching 0°C, mixture was stirred for 30 minutes, then recooled to −78°C. To the reaction mixture was added crude (*E*,*E*)-farnesal (**9**) (5.50 g, 25 mmol) in THF (25 ml). The resulting solution was warmed to room temperature, then stirred for 2 hours before being quenched by the addition of MeOH (1 ml). The resulting mixture was concentrated, then diluted with Et_2O and *n*-hexane (1 : 1 mixture). Removal of most of the triphenyl phosphine oxide by filtration through silica gel, followed by evaporation of the filtrate under reduced pressure, afforded 5.51 g of a yellow oil. Purification by chromatography on silica gel (*n*-hexane) furnished **10** as a colorless oil (5.40 g, 99%): bp 100~101°C; IR ν_{max} ($CHCl_3$) cm^{-1} 2900, 1450, 1380, 900; ^1H NMR (400 MHz, $CDCl_3$) δ 1.60 (6H, s), 1.68 (3H, d, J=1.0 Hz), 1.77 (3H, d, J=1.0 Hz), 1.96~2.16 (8H, m), 4.98 (1H, dd, J=1.5, 10.0 Hz), 5.09 (1H, dd, J=1.5, 10.0 Hz), 5.08~5.13 (2H, m), 5.86 (1H, d, J=11.0 Hz), 6.58 (1H, ddd, J=10.0, 11.0, 16.5 Hz); MS *m/z* 218 (M^+).

(3*E*,7*E*)-4,8,12-Trimethyl-3,7,11-tridecatrien-1-ol (Homofarnesol) (**11**)

To a solution of borone-tetrahydrofuran complex (1.0 M-THF solution, 33.7 ml, 33.7 mmol) was added 2-methyl-2-butene (2.0 M-THF solution, 36.7 ml, 73.4 mmol) at −30°C. The solution was warmed to 0°C and stirred for 2 hours. To the resulting mixture was added a solution of **10** (6.68 g, 30.6 mmol) in THF (15 ml) at 0°C. The reaction mixture was stirred for 2 hours at 0°C and was then warmed to room temperature, where it was allowed to remain for an additional 12 hours. Upon cooling the contents to −10°C, 3 N NaOH (3 ml) and 30% hydroperoxide (12 ml) were added to the reaction mixture. The solution was allowed to remain at room temperature for 3 hours. The mixture was then poured into water and extracted with EtOAc. The organic layer was washed with water and brine, and dried over anhydrous $MgSO_4$. Evaporation of the EtOAc under reduced pressure yielded a colorless oil which was purified by chromatography on silica gel (*n*-hexane-CH_2Cl_2, 1 : 3~1 : 5), to give the product **11** as a colorless oil (6.71 g, 93%): IR ν_{max} ($CHCl_3$) cm^{-1} 3600, 2920, 1440, 1380; ^1H NMR (400 MHz, $CDCl_3$) δ 1.60 (6H, s), 1.65 (3H, s), 1.68 (3H, s), 1.96~2.13 (8H, m), 2.29 (2H, q, J=6.5 Hz), 3.62 (2H, tr, J=6.5 Hz), 5.07~5.15 (3H, m); MS *m/z* 236 (M^+).

(3*E*,7*E*)-1-Bromo-4,8,12-trimethyl-3,7,11-tridecatriene (Homofarnesyl Bromide) (**12**)

To a solution of PPh_3 (2.20 g, 8.4 mmol) in CH_2Cl_2 (15 ml) was added Br_2 (0.5 ml) dropwise at 0°C until a permanent yellow color was achieved, then a small amount of PPh_3 and pyridine (1.3 ml, 16 mmol) was added. The mixture was stirred for 10 minutes, added to the solution of **11** (1.80 g, 7.5 mmol) in CH_2Cl_2 (10 ml) and then stirred for 1.5 hours at 0°C. After evaporation of the solvent, *n*-hexane was added to the residue. The resulting precipitate was removed *via* filtration through Celite. The filtrate was washed with 10% HCl and brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification of the crude product by chromatography on silica gel (*n*-hexane), furnished **12** as a colorless oil (2.17 g, 97%): IR ν_{max} ($CHCl_3$) cm^{-1} 2920, 1440, 1380; ^1H NMR (400 MHz, $CDCl_3$) δ 1.60 (6H, s), 1.63 (3H, s), 1.68 (3H, s), 1.96~2.12 (8H, m), 2.57 (2H, q, J=7.0 Hz), 3.34 (2H, tr, J=7.0 Hz), 5.07~5.16 (3H, m); MS *m/z* 298 (M^+).

Dimethyl (1*Z*,5*E*,9*E*)-6,10,14-trimethyl-1,5,9,13-pentadecatetraene-1,2-dicarboxylate (**15**)

To magnesium powder (204 mg, 8.5 mmol) (activated by washing with 0.3 M HCl, water, EtOH, and Et_2O successively, then drying overnight *in vacuo*)[21] in Et_2O (1 ml) at room temperature was added **12** (834 mg, 2.8 mmol) and dibromoethane (0.23 ml, 2.7 mmol) in Et_2O (3 ml) dropwise *via* syringe pump over 1 hour. The mixture was stirred at room temperature for 1 hour and then transferred *via* cannula into a suspension of CuBr·SMe_2 (626 mg, 3.0 mmol) in THF (2 ml) at −40°C. Following this addition, the reaction mixture was stirred for 50 minutes at the same temperature, then cooled to −78°C. To the resulting solution freshly distilled **14** (256 mg, 1.8 mmol) in THF (3 ml) was added dropwise. The reaction mixture was stirred at −78°C for 50 minutes, then poured into a saturated NH_4Cl aqueous solution. The mixture was partitioned with EtOAc, the organic phase was collected, and the aqueous phase was extracted with EtOAc. The combined organic extracts were washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification by chromatography on silica gel (*n*-hexane-EtOAc, 10 : 1), provided **15** as a colorless oil (494 mg, 76%): IR ν_{max} ($CHCl_3$) cm^{-1} 2920, 1720, 1440, 1280; ^1H NMR (400 MHz, $CDCl_3$) δ 1.59~1.60 (9H, m), 1.68 (3H, d, J=1.0 Hz), 1.95~2.10 (8H, m), 2.20 (2H, q, J=7.0 Hz), 2.38 (2H, tr, J=7.0 Hz), 3.73 (3H, s), 3.83 (3H, s), 5.07~5.11 (3H, m), 5.82 (1H, tr, J=1.5 Hz); MS *m/z* 362 (M^+).

(1*Z*,5*E*,9*E*)-6,10,14-Trimethyl-1,5,9,13-pentadecatetraene-1,2-dicarboxylic Acid (**16**)

To a solution of dimethyl ester **15** (215 mg, 0.59 mmol) in THF (25 ml) was added a solution of lithium hydroxide (142 mg, 5.9 mmol) in water (5.9 ml). Stirring continued at 40°C for 72 hours before concentrating *in vacuo*. To the aqueous residue was added 1 N HCl solution (6.0 ml, 6.0 mmol) at 0°C. The product was extracted with EtOAc, washed with brine, and dried over anhydrous $MgSO_4$. Evaporation of EtOAc under reduced pressure afforded a single product **16** as a colorless oil (190 mg, 97%): IR ν_{max} ($CHCl_3$) cm^{-1} 2920, 1775, 1720, 1240; ^1H NMR (400 MHz, $CDCl_3$) δ 1.60 (6H, s), 1.62 (3H, s), 1.68 (3H, s), 1.98~2.07 (8H, m), 2.26 (2H, q, J=7.0 Hz), 2.45 (2H,

tr, $J=7.0$ Hz), 5.08~5.17 (3H, m), 5.91(1H, s); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 16.0, 16.1, 17.7, 25.6, 25.7, 26.5, 26.7, 34.4, 39.7 (×2), 120.1, 121.6, 124.0, 124.3, 131.3, 135.1, 137.4, 150.8, 170.4, 174.1; MS *m/z* 316 ($M^+ - H_2O$).

Anal Calcd for $C_{20}H_{31}O_4$: C 71.82, H 9.04.
Found: C 72.09, H 9.04.

Acknowledgments

The author (HK) thanks Prof. F. E. MCDONALD (Northwestern University) for helpful suggestions and discussions.

References

1) For the review of squalene synthase inhibitor: ABE, I.; J. C. TOMESCH, S. WATTANASIN & G. D. PRESTWICH: Inhibitors of squalene biosynthesis and metabolism. Nat. Prod. Rep. 11: 279~302, 1994
2) Preliminary communication of this work has been published; TANIMOTO, T.; Y. TSUJITA, K. HAMANO, H. HARUYAMA, T. KINOSHITA, T. HOSOYA, S. KANEKO, K. TAGO & H. KOGEN: Schizostatin, a potent squalene synthase inhibitor from *Schizophyllum commune*: Isolation, structure elucidation, and total synthesis. Tetrahedron Lett. 36: 6301~6304, 1995
3) TANIMOTO, T.; K. ONODERA, T. HOSOYA, Y. TAKAMATSU, T. KINOSHITA, K. TAGO, H. KOGEN, T. FUJIOKA, K. HAMANO & Y. TSUJITA: Schizostatin, a novel squalene synthase inhibitor produced by the mushroom, *Schizophyllum commune*. I. Taxonomy, fermentation, isolation, physico-chemical properties, and biological activity. J. Antibiotics 49: 617~623, 1996
4) ADAMS, J. & M. L. GROSS: Tandem mass spectrometry for collisional activation of alkali-Metal-cationized fatty acids: A method for determining double bond location. Anal. Chem. 59: 1576~1582, 1987
5) TOMER, K. B.; F. W. CROW & M. L. GROSS: Location of double bond position in unsaturated fatty acids by negative ion MS/MS. J. Am. Chem. Soc. 105: 5487~5488, 1983
6) TAMAOKA, J.; K. KOMAGATA, T. KINOSHITA, G.-J. SHEN, T. KOMADA, T. IMAI & Y. MINODA: Methylubiquinone, a new isoprenoid quinone in methane-oxidizing bacterium strain H-2. FEMS. Microbiol. Lett. 29: 151~154, 1985
7) KITAOKA, M.; H. NAGAKI, T. KINOSHITA, M. KURABAYASHI, T. KOYAMA & K. OGURA: Negative ion fast atom bombardment-tandem mass spectrometry for structural analysis of isoprenoid diphosphates. Anal. Biochem. 185: 182~186, 1990
8) NAKAMURA, T.; T. TAKAZAWA, Y. MARUYAMA-OHKI, H. NAGAKI & T. KINOSHTA: Location of double bonds in unsaturated fatty alcohols by microderivatization and liquid secondary ion tandem mass spectrometry. Anal. Chem. 65: 837~840, 1993
9) SHIMAMURA, O.; Y. TAKAI, H. YAMADA, T. ISHIKAWA, M. SAWADA, S. TAKAHASHI, K. GOTO & T. KINOSHITA: Identification of unsaturated fatty acid amides involved in talc by FAB MS/MS measurements. J. Mass Spectrum. Soc. Jpn. 41: 159~163, 1993
10) COREY, E. J. & W.-C. SHIEH: A simple synthetic process for the elaboration of oligoprenols by stereospecific coupling of di-, tri-, or oligoisoprenoid units. Tetrahedron Lett. 33: 6435~6438, 1992
11) COREY, E. J.; M. C. NOE & W.-C. SHIEH: A short and convergent enantioselective synthesis of (3*S*)-2,3-oxidosqualene. Tetrahedron Lett. 34: 5995~5998, 1993
12) YANAGISAWA, A.; H. HIBINO, S. HABAUE, Y. HISADA & H. YAMAMOTO: Highly selective homocoupling reaction of allylic halides using barium metal. J. Org. Chem. 57: 6386~6387, 1992
13) YANAGISAWA, A.; H. HIBINO, S. HABAUE, Y. HISADA & H. YAMAMOTO: Regio- and stereoselective synthesis of 1,5-dienes using allylic barium reagents. Bull. Chem. Soc. Jpn. 68: 1263~1268, 1995
14) YANAGISAWA, A.; S. HABAUE & H. YAMAMOTO: Allylbarium in organic synthesis: Unprecedented α-selective and stereospecific allylation of carbonyl compounds. J. Am. Chem. Soc. 113: 5893~5895, 1991
15) YANAGISAWA, A.; S. HABAUE & H. YAMAMOTO: Direct insertion of alkali (alkaline-earth) metals into allylic carbon-halogen bonds avoiding stereorandomization. J. Am. Chem. Soc. 113: 8955~8956, 1991
16) YANAGISAWA, A.; S. HABAUE, K. YASUE & H. YAMAMOTO: Allylbarium reagents: Unprecedented regio- and stereoselective allylation reactions of carbonyl compounds. J. Am. Chem. Soc. 116: 6130~6141, 1994
17) WELCH, S. C. & J. M. GRUBER: Synthesis of C-13-substituted acid analogues. J. Org. Chem. 47: 385~389, 1982
18) NISHIYAMA, H.; M. SASAKI & K. ITO: Stereoselective addition of readily available organocopper reagents to dimethyl acetylenedicarboxylate. Chem. Lett.: 905~908, 1981
19) DODD, D. S. & A. C. OEHLSCHLAGER: Synthesis of inhibitors of 2,3-oxidosqualene-lanosterol cyclase: Conjugate addition of organocuprates to *N*-(carbobenzyloxy)-3-carbomethoxy-5,6-dihydro-4-pyridone. J. Org. Chem. 57: 2794~2803, 1992
20) LEOPOLD, E.: Selective hydroboration of a 1,3,7-triene: Homogeraniol: J. Org. Synth. 64: 164~174, 1986
21) KOCIEŃSKI, P. J.; C. J. LOVE, R. J. WHITBY, G. COSTELLO & D. A. ROBERTS: A total synthesis of (±)-zoapatanol and demethyl-ORF-13811. Tetrahedron 45: 3839~3841, 1989