Pergamon

Tetrahedron Letters, Vol. 36, No. 35, pp. 6301-6304, 1995
Elsevier Science Ltd
Printed in Great Britain
0040-4039/95 $9.50+0.00

0040-4039(95)01265-6

Schizostatin, a Potent Squalene Synthase Inhibitor from *Schizophyllum commune* : Isolation, Structure Elucidation, and Total Synthesis†

Tatsuo Tanimoto,[a] Yoshio Tsujita,[a] Kiyoshi Hamano,[b] Hideyuki Haruyama,[c] Takeshi Kinoshita,[c] Tsuyoshi Hosoya,[d] Satoru Kaneko,[e] Keiko Tago,[e] and Hiroshi Kogen*,[e]

[a] Pharmacology and Molecular Biology Research Laboratories, [b] Biomedical Research Laboratories, [c] Analytical and Metabolic Research Laboratories, [d] Tsukuba Research Laboratories, and [e] Exploratory Chemistry Research Laboratories
Sankyo Co., Ltd., 1-2-58 Hiromachi, Shinagawa, Tokyo 140, Japan

Abstract : The novel schizostatin (**1**) has been isolated as a potent inhibitor of squalene synthase. Its structure elucidation and total synthesis are described. Synthesis of the Z-isomer **12** and its biological activity are also reported.

A high level of cholesterol in the blood is considered to be one of the most important risk factors for atherosclerosis and coronary heart disease, and suppression of serum cholesterol levels is a precautionary measure for these diseases. *De novo* cholesterol biosynthesis inhibitors, specifically 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors such as pravastatin,[1] are effective therapeutic agents.

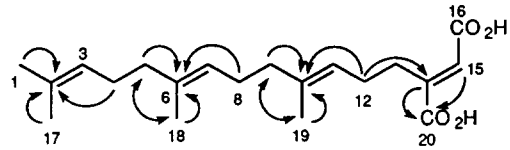
Squalene synthase (farnesyl diphosphate : farnesyl diphosphate transferase, EC 2.5.1.21)[2] is the enzyme which catalyzes the dimerization of farnesyl pyrophosphate to squalene, the first committed step to cholesterol in the isoprenoid biosynthetic pathway. More specifically the enzyme catalyzes a two-step conversion of two molecules of farnesyl pyrophosphate initially to presqualene pyrophosphate and subsequently to squalene. Specific and potent squalene synthase inhibitors have the potential to serve as useful cholesterol-lowering agents without adversely affecting the synthesis of other physiologically important isoprenoids such as dolichol, ubiquinone, isopentenyl tRNA, and farnesylated proteins.

Recently our screening efforts have concentrated on searching for inhibitors of squalene synthase. Indeed our efforts paid dividends, wherein isolation of schizostatin (**1**) as a fungal metabolite from mushroom *Schizophyllum commune* Fr. (SANK17785)[3] and testing its activity towards squalene synthase revealed its overwhelmingly strong competitive inhibitory activity (IC_{50} = 0.84μM). Inhibition of farnesyl protein transferase activity was not observed at high concentrations of schizostatin (IC_{50} > 300μM), suggesting that schizostatin showed specific inhibition towards squalene synthase.

Schizostatin (**1**)[4] has the molecular formula $C_{20}H_{31}O_4$ based on high-resolution mass spectral data (HREIMS, m/z 335.2205; Δ -1.8 mmu). The alkali-metal cationization method for the FAB-MS analysis of polyunsaturated acids[5] indicated the presence of unsaturated dicarboxylic acid moieties. The positive-ion FAB-MS/MS spectrum of **1** using the 3-nitrobenzylalcohol matrix containing lithium iodide gave three strong peaks of $(M + 1)^+$, $(M–H + 2Li)^+$ and $(M–2H + 3Li)^+$ ions, suggestive of **1** bearing two carboxylic acid functions. The FAB-MS spectrum of the $(M + Li)^+$ ion of **1** showed clear charge-remote fragmentation

†Dedicated to Prof. Tozo Fujii on the occasion of his 65th birthday.

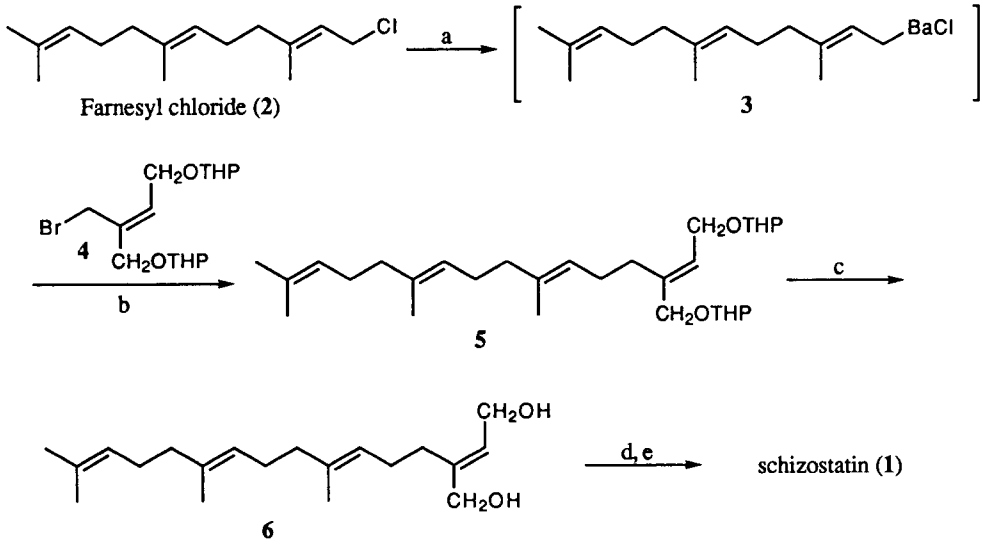
patterns[6] which was diagnostic of the olefinic positioning depicted in **1**. The ^{1}H and ^{13}C NMR spectra indicated the presence of four olefinic methyls, four double bonds, four olefinic protons, and two carbonyl groups. Scrutiny of the ^{1}H and ^{13}C long range coupling featured in a HMBC spectrum led us to both identify a dicarboxylic acid moiety derived from the first isoprene unit and the other three isoprene units. The stereochemistry at C3, C7, and C11 was assigned as *E* from their characteristic chemical shifts pertinent to this type of terpene structure. The stereochemistry at C15 was determined as *E* by a $^{13}C\{^{1}H\}$ NOE experiment, wherein the enhancement of C20 was observed along with C14 and C16 on irradiating H15 as shown in Fig. 1. Further confirmation of this was found in the actual synthesis of schizostatin (**1**) (*vide infra*).



Schizostatin (**1**)

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

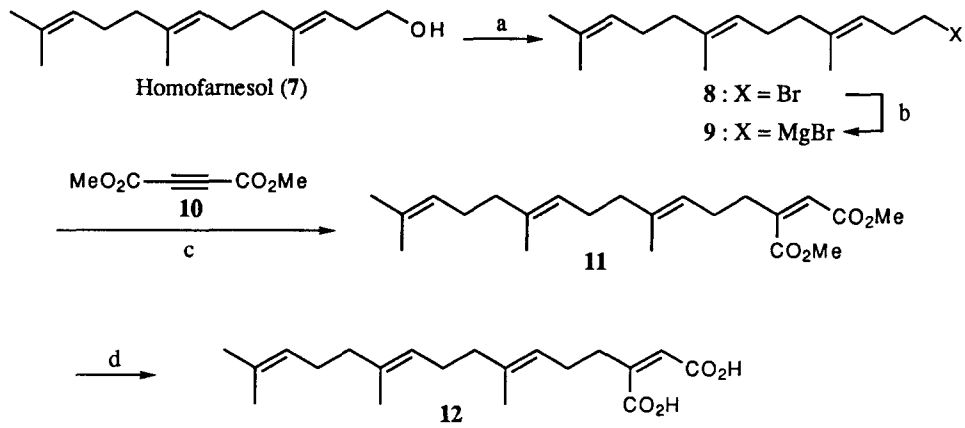
A highly stereocontrolled total synthesis of schizostatin (**1**) was accomplished by following the work of Corey[7] and Yamamoto[8] in which establishing the stereochemistry of the trisubstituted dicarboxylic acid moiety was attainable by employing an organobarium reagent[9] as shown in Scheme 1. Thus commercially available farnesyl chloride (**2**) was treated with two equivalents of lithium napthalenide and barium iodide in THF at room temperature; subsequent reaction with allyl bromide **4**[10] at –78°C gave conveniently coupling product **5** in 71% yield as a single regio- and stereoisomer. Deprotection of compound **5** with a catalytic amount of pyridinium *p*-toluenesulfonate (PPTS) in methanol at room temperature provided the diol **6** in 88%



Scheme 1 ; a) BaI_2, 2eq. lithium biphenylide, THF, r.t.; b) **4**, THF, -78°C; c) cat. PPTS, MeOH, r.t.; d) 2.6eq. $(COCl)_2$, Me_2SO, Et_3N, CH_2Cl_2, -78°C to r.t.; e) 2.5eq. $NaClO_2$, 20eq. NaH_2PO_4, 2-methyl-2-butene, t-BuOH, H_2O, 0°C.

yield. Oxidation of **6** in a sequential fashion using Swern and $NaClO_2$ conditions afforded schizostatin (**1**) in 72% yield after purification by reverse-phase Lobar® column chromatography (LiChroprep® RP-18, CH_3CN-H_2O, 65 : 35). Synthetic **1** was spectroscopically identical in all respects to that of the natural schizostatin sample (HPLC, ^1H and ^{13}C NMR, IR, MS, and melting point).

Synthesis of the *Z*-isomer **12** was initiated in order to evaluate its biological activity. Due to the instability of the *cis*-isomer of allylic bromide **4**, a different strategy featuring stereoselective *syn*-addition of an organocopper reagent[11] to dimethyl acetylenedicarboxylate (**10**) was applied as means to synthesizing the *Z*-isomer **12** as shown in Scheme 2. Henceforth homofarnesol (**7**)[12] was treated with triphenylphosphine and bromine in the presence of pyridine to give homofarnesyl bromide (**8**) in 97% yield. The bromide **8** was converted to Grignard reagent **9** by treating with magnesium in the presence of 1, 2-dibromoethane in anhydrous Et_2O at room temperature. The alkyl copper-dimethyl sulfide complex generated from the Grignard reagent **9** with cuprous bromide-dimethyl sulfide complex in anhydrous THF at -40°C, reacted with dimethyl acetylenedicarboxylate (**10**) at -78°C to obtain selectively the *Z*-dicarboxylate **11** in 76% yield. The dicarboxylate **11** was saponified with LiOH to give the *Z*-isomer of schizostatin **12**[13] in 97% yield. Compound **12** (IC_{50} = 12μM) was found to be 15-fold less potent as a competitive inhibitor of squalene synthase than that of schizostatin.



Scheme 2 ; a) Ph_3P, Br_2, pyridine, CH_2Cl_2, 0°C; b) Mg, $BrCH_2CH_2Br$, Et_2O, r.t.; c) (i) CuBr•Me_2S, THF, -40°C; (ii) **10**, THF, -78°C; c) 10eq. LiOH, THF-H_2O, 40°C.

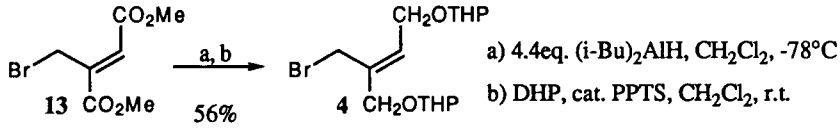
Acknowledgment : The author (HK) thanks Prof. F. E. McDonald (Northwestern University) for helpful suggestions and discussions.

References and Notes

1. Furberg, C. D.; Byington, R. P.; Crouse, J. R.; Espeland, M. A. *Am. J. Cardiol.*, **1994**, *72*, 1133-1134 and references cited therein.
2. For the review of squalene synthase inhibitor : Abe, I.; Tomesch, J. C.; Wattanasin, S.; Prestwich, G. D. *Nat. Prod. Rep.*, **1994**, *11*, 279-302.
3. The culture filtrate of this fungus was adjusted to pH 3.5 with conc. HCl and then extracted with ethyl acetate. The oily substance obtained was purified by preparative reverse phase

HPLC (YMC-pack C-18 column, 1.0% triethylamine-phosphoric acid buffer (pH 3.2)-CH_3CN (30/70)) give schizostatin (**1**).

4. Schizostatin (**1**) : ^1H NMR ($CDCl_3$, 400MHz): δ 1.60 (6H, s), 1.61 (3H, s), 1.68 (3H, s), 1.95–2.10 (8H, m), 2.23 (2H, q, J = 7.5Hz), 2.86 (2H, t, J = 7.5Hz), 5.07–5.14 (2H, m), 5.18 (1H, t, J = 7.0Hz), 6.89 (1H, s). ^{13}C NMR ($CDCl_3$, 100MHz): δ 15.9, 16.0, 17.7, 25.7, 26.6, 26.8, 27.6, 27.9, 39.7 (x 2), 122.5, 124.1, 124.4, 127.6, 131.2, 135.1, 137.0, 149.1, 171.0, 172.3 ppm. mp 119–122°C. IR ($CHCl_3$): 1705, 1695, 1410 cm^{-1}.
5. Adams, J.; Gross, M. L. *Anal. Chem.* **1987**, *59*, 1576-1582.
6. a) Tomer, K. B.; Crow, F. W.; Gross, M. L. *J. Am. Chem. Soc.* **1983**, *105*, 5487-5488, b) Tamaoka, J.; Komagata, K.; Kinoshita, T.; Shen, G.-J.; Komada, T.; Imai, T.; Minoda, Y. *FEMS Microbiol. Lett.* **1985**, *29*, 151-154, c) Kitaoka, M.; Nagaki, H.; Kinoshita, T.; Kurabayashi, M.; Koyama, T.; Ogura, K. *Anal. Biochem.* **1990**, *185*, 182-186, d) Nakamura, T.; Takazawa, T.; Maruyama-Ohki, Y.; Nagaki, H.; Kinoshita, T. *Anal. Chem.* **1993**, *65*, 837-840, e) Shimamura, O.; Takai, Y.; Yamada, H.; Ishikawa, T.; Sawada, M.; Takahashi, S.; Goto, K.; Kinoshita, T. *J. Mass Spectrum. Soc. Jpn.* **1993**, *41*, 159-163.
7. a) Corey, E. J.; Shieh, W.-C. *Tetrahedron Lett.* **1992**, *33*, 6435-6438, b) Corey, E. J.; Noe, M. C. ; Shieh, W.-C. *ibid.* **1993**, *34*, 5995-5998
8. a) Yanagisawa, A.; Hibino, H.; Habaue, S.; Hisada, Y.; Yamamoto, H. *J. Org. Chem.* **1992**, *57*, 6386-6387, b) Yanagisawa, A.; Hibino, H.; Habaue, S.; Hisada, Y.; Yamamoto, H. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 1263-1268.
9. a) Yanagisawa, A.; Habaue, S.; Yamamoto, H. *J. Am. Chem. Soc.* **1991**, *113*, 5893-5895; 8955-8956, b) Yanagisawa, A.; Habaue, S.; Yasue, K.; Yamamoto, H. *J. Am. Chem. Soc.* **1994**, *116*, 6130-6141
10. Allylic bromide **4** was prepared in two steps from readily available 2-bromomethyl-2-butene-1, 4-dioic acid dimethyl ester (**13**) as shown below. For preparation of **13**, see Welch, S. C.; Gruber, J. M. *J. Org. Chem.* **1982**, *47*, 385-389.



11. Nishiyama, H.; Sasaki, M.; Ito, K. *Chem. Lett.* **1981**, 905-908.
12. Homofarnesol was prepared in 3 steps from farnesol. Leopold, E. J. *Org. Synth.* **1986**, *64*, 164-174.
13. **12** : ^1H NMR ($CDCl_3$, 400MHz): δ 1.60 (6H, s), 1.62 (3H, s), 1.68 (3H, s), 1.98–2.07 (8H, m), 2.26 (2H, q, J = 7.0Hz), 2.45 (2H, t, J = 7.0Hz), 5.08–5.17 (3H, m), 5.91 (1H, s). ^{13}C NMR ($CDCl_3$, 67.5MHz): δ 16.0, 16.1, 17.7, 25.6, 25.7, 26.5, 26.7, 34.4, 39.7 (x 2), 120.1, 121.6, 124.0, 124.3, 131.3, 135.1, 137.4, 150.8, 170.4, 174.1 ppm. IR ($CHCl_3$): 2920, 1775, 1720, 1240 cm^{-1}.

(*Received in Japan* 5 *June* 1995; *revised* 3 *July* 1995; *accepted* 4 *July* 1995)