VOL. 54 NO. 12, DEC. 2001 THE JOURNAL OF ANTIBIOTICS pp. 1025 — 1030

CJ-13,981 and CJ-13,982, New Squalene Synthase Inhibitors

SHUZO WATANABE*, HIDEO HIRAI, TAKAHITO KAMBARA, YASUHIRO KOJIMA, HIROYUKI NISHIDA, AYUMU SUGIURA, YUJI YAMAUCHI, NOBUJI YOSHIKAWA, H. JAMES HARWOOD, Jr.†, LIANG HSIUNG HUANG† and NAKAO KOJIMA††

Exploratory Medicinal Sciences, PGRD, Nagoya Laboratories, Pfizer Pharmaceuticals Inc., 5-Gochi 2, Taketoyo-cho, Chita-gun, Aichi 470-2393, Japan
† PGRD, Groton Laboratories, Pfizer Inc., Eastern Point Road, Groton, CT 06340, USA
†† Faculty of Pharmacy, Meijo University, 150 Yagotoyama, Tempaku-ku, Nagoya 468-8503, Japan

(Received for publication June 21, 2001)

Two new squalene synthase (SSase) inhibitors, CJ-13,981 (**I**) and CJ-13,982 (**II**), were isolated from the fermentation broth of an unidentified fungus CL15036. They inhibited human liver microsomal SSase with IC_{50}s of 2.8 and 1.1 μM, respectively, but showed no inhibitory activity against human brain protein farnesyltransferase (PFTase) at 100 μM. Based on FAB-MS and NMR analyses, the structures of **I** and **II** were determined to be 3-hydroxy-3,4-dicarboxy-15-hexadecenoic acid and 3-hydroxy-3,4-dicarboxyhexadecanoic acid, respectively.

Squalene synthase (SSase, EC 2.5.1.21) occupies a key branch-point in the isoprenoid pathway, catalyzing the first committed step of the *de novo* sterol biosynthesis. SSase catalyzes the reductive dimerization of two molecules of farnesyl pyrophosphate to form squalene *via* the intermediate, presqualene pyrophosphate. SSase is an attractive target for pharmacological intervention to reduce serum cholesterol levels because the inhibition at this step would not influence the biosynthesis of other essential non-sterol products, such as ubiquinone, dolichol, isopentenyl t-RNA and prenylated proteins[1,2].

In the course of our screening program for discovery of SSase inhibitors, an unidentified fungus CL15036 was found to produce two new SSase inhibitors, CJ-13,981 (**I**) and CJ-13,982 (**II**). In this paper we report the fermentation, isolation, physico-chemical properties, structure elucidation and biological activities of **I** and **II**.

Results

Taxonomy

On media such as cornmeal agar, Czapek-sucrose agar, malt extract agar, oatmeal agar, and potato dextrose agar, the fungus is characterized by the pale yellowish, pale orange-pink, pale pinkish buff, light buff to tan colonies; the pale yellowish, pale pinkish orange, pale orange, pale orange-buff, pale buff, pale gray, gray to black colony reverse; and lack of soluble pigment. Growth was moderate at 20°C, moderate to good at 28°C, good to excellent at 37°C, and none at 45°C and 50°C. The hyphae were septate and branched, and hyaline initially, but became tan, pink buff or ochreous buff. They might become brown, gray or black after a long incubation of 4 to 5 weeks or upon exposure to fluorescent light or black light. On several media chlamydospores of various shapes ranging from globose, subglobose, oval, elliptical, to irregular were produced. No sexual spores or conidia were produced on any of the media used.

On the basis of the above, the fungus can not be identified to a fungal genus because of the absence of sexual spores or conidia necessary for identification and thus is considered as an unidentified fungus.

* Corresponding author: Shuzo.Watanabe@japan.pfizer.com

Table 1. Physico-chemical properties of CJ-13,981 (**I**) and CJ-13,982 (**II**).

	CJ-13,981 (**I**)	CJ-13,982 (**II**)
Appearance	White amorphous powder	White amorphous powder
$[\alpha]_D$ (26°C)	-18.52° (*c* 0.81, acetone)	-18.34° (*c* 2.6, acetone)
Molecular formula	$C_{18}H_{30}O_7$	$C_{18}H_{32}O_7$
Molecular weight	358	360
HRFAB-MS (*m/z*)		
Found :	357.1905 $(M-H)^-$	359.2073 $(M-H)^-$
Calcd. :	357.1913 (for $C_{18}H_{29}O_7$)	359.2067 (for $C_{18}H_{31}O_7$)
UV λ_{max} (nm, MeOH)	215	215
IR ν_{max} (cm^{-1}, KBr)	3490, 2915, 2855, 1694 1417, 1263, 918	3480, 2915, 2855, 1698 1414, 1261, 945
Solubility		
Soluble:	MeOH, DMSO	MeOH, DMSO
Insoluble:	H_2O	H_2O

Fermentation

An unidentified fungus CL15036 was maintained on a potato dextrose agar slant (Difco). A vegetative cell suspension from the slant was used to inoculate a 500-ml Erlenmeyer flask containing 100 ml of a seed medium (potato dextrose broth 2.4%, yeast extract 0.5% and agar 0.1%). The flask was shaken at 26°C for 4 days on a rotary shaker (7-cm throw at 210 rpm). Five ml aliquot was inoculated into a 500-ml Erlenmeyer flask containing 150 ml of the seed medium, and shaken at 26°C for 4 days. The second seed culture was inoculated to a 6-liter jar fermentor containing 3 liters of a production medium (potato dextrose broth 2.4%, yeast extract 0.5% and agar 0.1%). The fermentation was carried out at 26°C for 9 days at an aeration rate of 3 liters/minute and an agitation rate of 1,700 rpm.

Isolation

The detection of **I** and **II** was monitored by HPLC using an ODS column as described in the experimental section. The fermentation broth (3 liters) was filtered after the addition of 2 liters of EtOH. The filtrate was concentrated to an aqueous solution (1 liter) and adjusted to pH 3 with 1 M HCl, and was extracted 3 times with 1 liter of EtOAc. The EtOAc layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The resulting residue (3.5 g) was applied to preparative HPLC on an ODS column (YMC-pack ODS AM-343, 20×250 mm, YMC Co., Ltd.) with MeOH - 0.1% TFA in H_2O (85 : 15) at a flow rate of 10 ml/minute. The eluted peaks were collected and concentrated to yield **I** (198 mg) and **II** (508 mg) as white powder.

Physico-chemical Properties

The physico-chemical properties of **I** and **II** are summarized in Table 1. They were obtained as amorphous white powder and were soluble in MeOH and DMSO, but insoluble in H_2O. The IR spectra exhibited the presence of a hydroxyl (3500 cm^{-1}) and a carbonyl (1700 cm^{-1}) group.

Structure Elucidation of CJ-13,981 (**I**)

The molecular formula of **I** was determined to be $C_{18}H_{30}O_7$ [*m/z* found: 357.1905, calcd. 357.1913 for $C_{18}H_{29}O_7$] by HRFAB-MS. The structure of **I** was elucidated based on the results of ^1H-^1H COSY and selective INEPT experiments as shown in Fig. 1. The ^1H-^1H COSY experiment revealed a proton sequence, $H_2C{=}CH{-}CH_2{-}(CH_2)_n{-}CH_2{-}CH{-}$. The methine proton of H-15 (δ 5.79) was coupled with the methylene protons of H-14 (δ 2.03) and H-16 (δ 5.01). The methylene proton of H-14 was coupled with the methylene protons at δ 1.14~1.40, although the coupling of methylene protons at δ 1.14~1.40 was not clarified because of the overlap of methylene signals. The methylene protons (δ 1.14~1.40) were coupled with the methylene protons of H-5 (δ 1.79 and 1.47), which also coupled with the methine proton of

Fig. 1. ^{1}H-^{1}H COSY and selective INEPT experiments of CJ-13,981 (**I**).

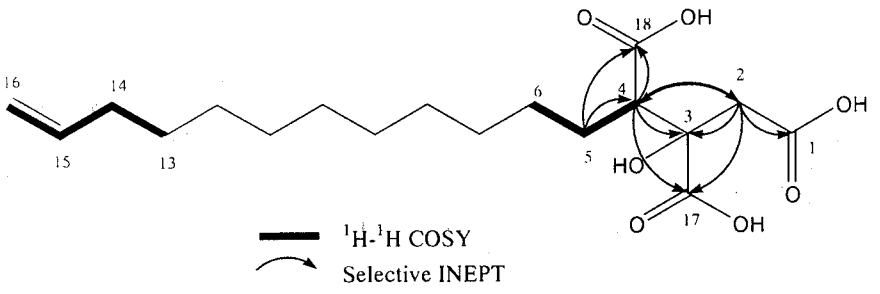
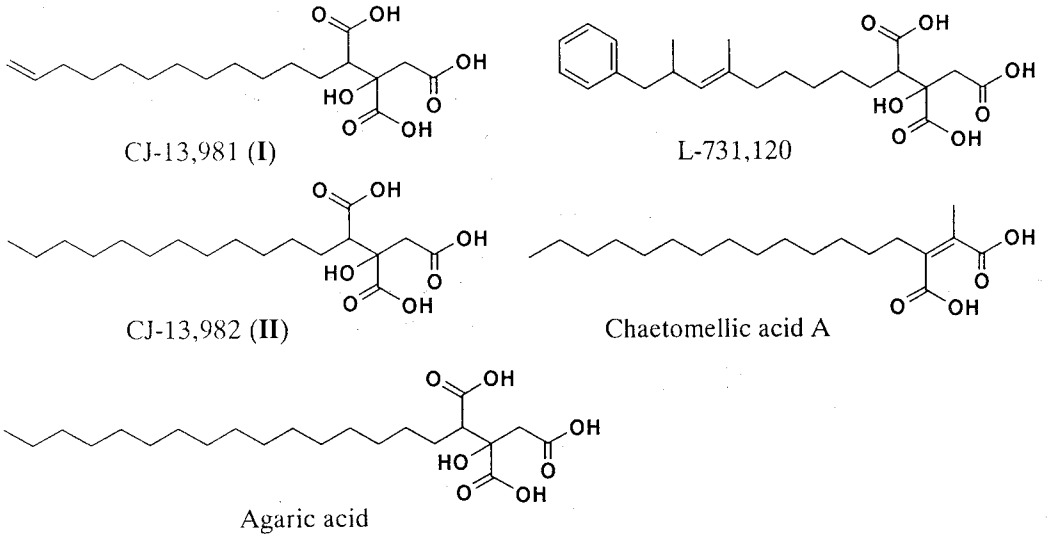


Fig. 2. Structures of CJ-13,981 (**I**), CJ-13,982 (**II**) and their related compounds.



H-4 (δ 2.64). The structure of 3,4-dicarboxy-3-hydroxybutanoic acid moiety was determined by the selective INEPT experiments. The selective INEPT spectra showed the following ^{1}H-^{13}C long range couplings: from H-2 (δ 2.68) to C-1 (δ 174.7), C-3 (δ 77.6), C-4 (δ 55.6) and C-17 (δ 177.4), from H-4 to C-2 (δ 43.1), C-3, C-17 and C-18 (δ 177.0), and from H-5 (δ 1.79) to C-4 and C-18. These data supported the 3,4-dicarboxy-3-hydroxybutanoic acid moiety. From the above data, the structure of **I** was determined as shown in Fig. 2.

Structure Elucidation of CJ-13,982 (**II**)

The structure of **II** was determined by a comparison of its spectral properties with those of natural agaric acid (Fig. 2)[3,4]. The ^{1}H and ^{13}C NMR spectra were particularly informative. The structure of **II** was considered to be very similar to that of agaric acid based on spectral data. The ^{1}H and ^{13}C NMR showed that loss of four methylene groups of the alkyl chain had occurred in this compound. The HRFAB-MS [*m/z* found: 359.2073, calcd. 359.2067 for $C_{18}H_{31}O_7$] also supported the loss of four methylene groups. From the above data, the structure of **II** was determined as shown in Fig. 2.

Biological Activities

As shown in Table 2, **I** and **II** inhibited both rat and human liver microsomal SSase with IC_{50}s that range from 1.1 to 4.2 μM. However, they did not inhibit cholesterol biosynthesis in either Hep-G2 cells (IC_{50} >100 μM) or chow-fed mice (ED50 >30 mg/kg, po). In addition, they

Table 2. Biological activities of CJ-13,981 (**I**) and CJ-13,982 (**II**).

Compound	SSase (IC_{50}, μM)		Cholesterol biosynthesis		Human brain PFTase
	Rat liver	Human liver	Hep-G2 cells (IC_{50}, μM)	Mice* (% inhibition)	(IC_{50}, μM)
CJ-13,981 (**I**)	4.2	2.8	>100	34	>100
CJ-13,982 (**II**)	2.2	1.1	>100	0	>100

* 30 mg/kg (po).

exhibited no inhibitory activity against human brain PFTase at 100 μM.

Discussion

Compounds **I** and **II** inhibited rat and human liver microsomal SSases, but exhibited no promising activity for the inhibition of cholesterol biosynthesis in Hep-G2 cells and in chow-fed mice. This difference suggests that poor absorption of these compounds may be a major issue, as was observed with other polyanionic compounds (*e.g.* zaragozic acids[5,6] and bisphosphonates[7]). In fact, an acyclic tricarboxylic compound, L-731,120[8], which is structurally similar to **I**, inhibits cholesterol biosynthesis in mice when administered subcutaneously (ED_{50}=15 mg/kg).

Compounds **I** and **II** showed a high specificity (>30-fold) for human liver microsomal SSase inhibition relative to human brain PFTase inhibition. PFTase catalyzes the transfer of the farnesyl group of farnesyl pyrophosphate to proteins ending with a carboxy-terminal CAAX (C: Cys; A: aliphatic amino acid; X: Ser or Met) motif[9,10]. As a structurally-related compound of **II**, chaetomellic acid A[11] isolated from a fungus *Chaetomella acutiseta*, was reported to be a specific human PFTase inhibitor that did not inhibit human SSase (IC_{50} >150 μM)[12]. The structural difference between **II** and chaetomellic acid A is that **II** has a citric acid moiety whereas chaetomellic acid A has a methylmaleic acid moiety. This suggests that, whereas SSase and PFTase both use farnesyl pyrophosphate as a substrate, the substrate-binding pocket on each enzyme can effectively discriminate between the polyanionic functions.

Experimental

General

Spectral and physico-chemical data were obtained on the following instruments: UV, JASCO Ubest-30; IR, Shimadzu IR-470; optical rotations, JASCO DIP-370 with a 10 cm cell; NMR, JEOL JNM-GX270 updated with an LSI-11/73 host computer, TH-5 tunable probe and version 1.6 software; and LRFAB- and HRFAB-MS, JEOL MS-700 with a mastation data processing system. All NMR spectra were measured in CD_3OD unless otherwise indicated, and peak positions are expressed in parts per million (ppm) based on the internal standard of the MeOH peak at 3.30 ppm for ^1H NMR and 49.8 ppm for ^{13}C NMR. All FAB-MS spectra were measured using glycerol-matrix.

Producing Microorganism

The producing strain CL15036 was an unidentified fungus designated as Z-285 from the Texas Technical University, Lubbock, Texas, USA, and deposited as FERM BP-5153 at the National Institute of Bioscience· and Human-Technology, Agency of Industrial Science and Technology (Tsukuba, Japan).

HPLC Analysis

HPLC analysis was performed on a Hewlett Packard HP1090 system. Samples were subjected to an ODS column (YMC-pack ODS AM-312, 6.0×150 mm, YMC Co., Ltd.) maintained at 42°C and eluted with MeOH-0.1% TFA in H_2O (85 : 15) at a flow rate of 0.8 ml/minute. Compounds **I** and **II** were monitored by absorbance at 220 nm. Under these conditions, **I** and **II** were eluted at the retention times of 7.0 and 8.7 minutes, respectively.

^{1}H and ^{13}C NMR Spectra

I: ^{1}H NMR (δ) 1.14~1.40 (16H, m), 1.47 (1H, m), 1.79 (1H, m), 2.03 (2H, m), 2.64 (1H, dd, J=2.9, 11.7 Hz), 2.68 (1H, d, J=16.5 Hz), 3.07 (1H, d, 16.5 Hz), 4.87~5.01 (2H, m) and 5.79 (1H, ddt, J=10.1, 17.0, 6.6 Hz); ^{13}C NMR (ppm) 28.89, 29.61, 30.91, 31.01, 31.24 (2C), 31.40 (2C), 31.46, 35.69, 43.08, 55.59, 77.60, 115.49, 140.94, 174.65, 176.97 and 177.41. **II**: ^{1}H NMR (δ) 0.89 (3H, t, J=6.5 Hz), 1.27 (20H, m), 1.49 (1H, m), 1.79 (1H, m), 2.64 (1H, m), 2.68 (1H, d, J=16.5 Hz), 3.08 (1H, d, J=16.5 Hz); ^{13}C NMR (ppm) 15.25, 24.52, 28.87, 29.60, 31.26 (3C), 31.44 (2C), 31.54 (3C), 33.86, 43.08, 55.57, 77.59, 174.68, 176.97 and 177.41.

Preparation of Rat Hepatic Microsomes

Livers freshly obtained from Wistar rats (Charles River) were rinsed in ice-cold PBS and briefly homogenized in buffer A (50 mM MOPS-NaOH; pH 7.4, 10 mM $MgCl_2$, 1 mM EDTA, 10 mM dithiothreitol) containing the protease inhibitors (500 μM PMSF, 10 μM aprotinin, 10 μM leupeptin, 10 μM chymostatin). The homogenate was centrifuged at 3,000×g for 20 minutes at 4°C. The supernatant was recentrifuged at 20,000×g for 30 minutes at 4°C. The supernatant was again centrifuged at 100,000×g for 60 minutes at 4°C. After centrifugation, the supernatant was removed, and the pellet was suspended in buffer A. This microsomal preparation had a protein concentration of about 13.2 mg/ml. The microsomal suspensions were stored at −70°C. Under this condition, SSase activity was stable for at least several months.

SSase Activity

Assays were performed on 96-well microtiter plates in a total volume of 50 μl containing 50 mM MOPS-NaOH (pH 7.4), 10 mM KF, 10 mM $MgCl_2$, 2 mM CHAPS, 10 mM dithiothreitol, 0.5 mM NADPH, 50 mM ascorbate, 20 units/ml ascorbate oxidase, 2 mM glucose-6-phosphate, 20 units/ml glucose-6-phosphate dehydrogenase, rat hepatic microsomes and 6 μM [1-^{3}H]FPP. After incubation at room temperature for 30 minutes, the reaction was terminated by the addition of 20 μl of 1-propanol containing unlabeled 1% squalene. Thirty microliters of reaction mixture were applied onto polyester-backed silica gel TLC sheets (Sigma; 10×10 cm) which were developed by n-hexane-EtOAc (7:3). After drying, radioactivity in zones containing the squalene (Rf=0.8) were scraped and measured by a scintillation counter[13,14].

PFTase Activity

Human brain cytosol used as the source of PFTase activity was prepared as previously described[13]. Inhibition of the catalytic activity of PFTase was evaluated by measuring inhibition of the covalent incorporation of the farnesyl moiety of [1-^{3}H]FPP into H-Ras using a procedure that combines room temperature protein precipitation with sample filtration and radioactivity assessment in a 96-well format using microplate liquid scintillation counting technology [final assay concentrations: 0.5 μM [1-^{3}H]FPP (sp. act. ~13,000 dpm/pmol), 5 mM $MgCl_2$, 20 μM $ZnCl_2$, 4 μM H-ras, 50 mM Tris HCl (pH=7.5), 5 mM DTT, 20 mM KCl, 1.2% DMSO, 1.2 mg/ml rat brain cytosolic protein][13]. PFTase activity is expressed as picomoles H-Ras farnesylated per minute of incubation at 37°C per mg cytosolic protein.

Cholesterol Synthesis Activity in Hep-G2 Cells

The rate of cholesterol synthesis in Hep-G2 cells was evaluated by measuring incorporation of [2-^{14}C]acetate into cholesterol and related sterols[14]. Hep-G2 cells were seeded in 24 well plates at a density of 1.2×10^5 cells/well and maintained in 1.0 ml of Dulbecco's Minimal Essential Medium containing 10% heat-inactivated fetal bovine serum 2 mM L-glutamine, and 40 μg/ml gentamicin for 7 days in a 37°C, 5% CO_2 incubator with medium changes on days 3 and 5. On day 8, at a time when cultures reached 80~90% confluency (late-log phase) and maintained a >90% cell viability, the medium was removed and replaced with fresh medium containing 1% DMSO±effector compounds. Immediately after compound addition, 25 μl of medium containing 4 μCi of [2-^{14}C]acetate (56 mCi/mmol) was added to each incubation well. Plates were then sealed with parafilm and incubated at 37°C for 6 hours with gentle shaking. After incubation, the samples were saponified by addition to each well of 1 ml of 5 M KOH in MeOH, followed first by incubation for 2 hours at 70°C and then by overnight incubation at room temperature. After saponification, the resulting mixtures were transferred to glass conical tubes and extracted three times with 4.5 ml n-hexane. The pooled organic fractions were dried under nitrogen, resuspended in 25 μl chloroform, and applied to 1×20 cm channels of silica gel 60C TLC plates (Eastman Kodak, Rochester NY). Channels containing non-radioactive cholesterol, lanosterol and squalene were included on selected TLC plates as separation markers. TLC plates were developed in n-hexane-diethyl ether-acetic acid (70:30:2), air-dried, and assessed for radioactivity using a Berthold Linear Radioactivity Analyzer that reports peak location and integrated peak area. Cholesterol synthesis is expressed as dpm [^{14}C]acetate incorporated into cholesterol during the 6 hours incubation

at 37°C.

In Vivo Cholesterol Synthesis Activity

Hepatic cholesterol synthesis in mice was evaluated by measuring incorporation of [2-^{14}C]mevalonate into cholesterol and related sterols as previously described for golden Syrian hamsters[15]. Male CD1 mice weighing about 30 g were administered a 0.2 ml per 40 g body weight oral bolus of vehicle±effector compound. One hour after compound administration, animals received an intraperitoneal injection of 0.1 ml *R,S*-[2-^{14}C]mevalonolactone (2 μCi/ml; 54.1 mCi/mmol). One hour after radiolabel administration, animals were euthanized by pentobarbital injection and two 1 g liver pieces were removed. Tissue samples were saponified at 70°C for 120 minutes in 2.5 ml of 2.5 M NaOH, then 4 ml of absolute EtOH were added to each sample and the solutions were mixed. Eight milliliters of petroleum ether were then added to each sample and the mixtures were shaken vigorously for 2 minutes then allowed stand until complete phase separation was achieved. The resultant petroleum ether layers were removed and 2.0 ml aliquots were mixed with 10 ml Ready-Safe liquid scintillation fluid and assessed for radioactivity (total nonsaponifiable lipid synthesis) using a Beckman LS6500 liquid scintillation counter. Cholesterolgenesis is expressed as dpm [2-^{14}C]mevalonolactone incorporated into nonsaponifiable lipids per gram liver during the 1 hour interval between [2-^{14}C]mevalonolactone injection and pentobarbital administration.

References

1) ABE, I.; J. C. TOMESCH, S. WATTANASIN & G. D. PRESTWICH: Inhibition of squalene biosynthesis and metabolism. Nat. Prod. Repots: 279~302, 1994
2) GOLDSTEIN, J. L. & M. S. BROWN: Regulation of the mevalonate pathway. Nature 343: 425~430, 1990
3) THOMS, H. & J. VOGELSANG: Zur Kenntniss der Agaricinsäure. Justus Liebigs Ann. Chem. 357: 145, 1907
4) BRANDÄNGE, S.; S. JOSEPHSON, L. MÖRCH & S. VALLÉN: Absolute configurations of alkylcitric acids. Acta Chem. Scand., Ser. B 31: 307, 1977
5) BAXTER, A.; B. J. FITZGERALD, J. L. HUTSON, A. D. MCCARTHY, J. M. MOTTERAM, B. C. ROSS, M. SAPRA, M. A. SNOWDEN, N. S. WATSON, R. J. WILLIAMS & C. WRIGHT: Squalestatin 1, a potent inhibitor of squalene synthase, which lowers serum cholesterol *in vivo*. J. Biol. Chem. 267: 11705~11708, 1992
6) BERGSTROM, J. D.; M. M. KURTZ, D. J. REW, A. M. AMEND, J. D. KARKAS, R. G. BOSTEDOR, V. S. BANSAL, C. DUFRESNE, F. L. VANMIDDLESWORTH, O. D. HENSENS, J. M. LIESCH, D. L. ZINK, K. E. WILSON, J. ONISHI, J. A. MILLIGAN, G. BILLS, L. KAPLAN, M. N. OMSTEAD, R. G. JENKINS, L. HUANG, M. S. MEINZ, L. QUINN, R. W. BURG, Y. L. KONG, S. MOCHALES, M. MOJENA, I. MARTIN, F. PELAEZ, M. T. DIEZ & A. W. ALBERTS: Zaragozic acids: a family of fungal metabolites that are picomolar competitive inhibitors of squalene synthase. Proc. Natl. Acad. Sci. USA 90: 80~84, 1993
7) CIOSEK, Jr., C. P.; D. R. MAGNIN, T. W. HARRITY, J. V. H. LOGAN, J. K. DICKSON, Jr., E. M. GORDON, K. A. HAMILTON, K. G. JOLIBOIS, L. K. KUNSELMAN, R. M. LAWRENCE, K. A. MOOKHTIAR, L. C. RICH, D. A. SLUSARCHYK, R. B. SULSKY & S. A. BILLER: Lipophilic 1,1-bisphosphonates are potent squalene synthase inhibitors and orally active cholesterol lowering agents *in vivo*. J. Biol. Chem. 268: 24832~24837, 1993
8) HARRIS, G. H.; H. JOSHUA & D. L. ZINK (Merck & Co., Inc.): Cholesterol lowering compounds. U.S. 5,286,895, Feb. 15, 1994
9) MALTESE, W. A.: Posttranslational modification of proteins by isoprenoids in mammalian cells. FASEB J. 4: 3319~3328, 1990
10) CLARKE, S.: Protein isoprenylation and methylation at carboxyl-terminal cysteine residues. Annu. Rev. Biochem. 61: 335~386, 1992
11) SINGH, S. B.; D. L. ZINK, J. M. LIESCH, M. A. GOETZ, R. G. JENKINS, M. NALLIN-OMSTEAD, K. C. SILVERMAN, G. F. BILLS, R. T. MOSLEY, J. B. GIBBS, G. ALBERS-SCHONBERG & R. B. LINGHAM: Isolation and structure of chaetomellic acids A and B from *Chaetomella acutiseta*: Farnesyl pyrophosphate mimic inhibitors of Ras farnesyl-protein transferase. Tetrahedron 49: 5917~5927, 1993
12) LINGHAM, R. B.; K. C. SILVERMAN, G. F. BILLS, C. CASCALES, M. SANCHEZ, R. G. JENKINS, S. E. GARTNER, I. MARTIN, M. T. DIEZ, F. PELÁEZ, S. MOCHALES, Y.-L. KONG, R. W. BURG, M. S. MEINZ, L. HUANG, M. NALLIN-OMSTEAD, S. D. MOSSER, M. D. SCHABER, C. A. OMER, D. L. POMPLIANO, J. B. GIBBS & S. B. SINGH: *Chaetomella acutiseta* produces chaetomellic acids A and B which are reversible inhibitors of farnesyl-protein transferase. Appl. Microbiol. Biotechnol. 40: 370~374, 1993
13) HARWOOD, Jr., H. J.: Measurement of enzymatic activity in 96-well format using TopCount microplate scintillation counting technology. Anal. Biochem. 226: 268~278, 1995
14) PETRAS, S. F.; S. LINDSEY & H. J. HARWOOD, Jr.: HMG-CoA reductase regulation: use of structurally diverse first half-reaction squalene synthetase inhibitors to characterize the site of mevalonate-derived nonsterol regulator production in cultured IM-9 cells. J. Lipid Res. 40: 24~38, 1999
15) HARWOOD, Jr., H. J.; E. G. BARBACCI-TOBIN, S. F. PETRAS, S. LINDSEY & L. D. PELLARIN: 3-(4-Chlorophenyl)-2-(diethylaminoethoxyphenyl)-A-pentonitrile monohydrogen citrate and related analogs: reversible, competitive, first half-reaction squalene synthetase inhibitors. Biochem. Pharmacol. 53: 839~864, 1997