Purification and Characterization of a Lovastatin Esterase from *Clonostachys compactiuscula*

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An esterase from the fungus *Clonostachys compactiuscula* selectively hydrolyzes lovastatin, a clinically useful antihypercholesterolemic agent. Lovastatin or lovastatin-related compounds were required to induce the activity of the lovastatin 8'-(α -methylbutyryloxy) esterase. The 46-kDa esterase was purified from mycelial extracts by centrifugation and a single anion-exchange chromatographic separation. Maximal lovastatin esterase activity was found at pH 9.0 to 9.6 and at 25 to 30°C. The addition of 5 to 20% methanol resulted in greater lovastatin hydrolysis, while the addition of other solvents (ethanol, isopropanol, butanol, ethyl acetate, isopropyl acetate, or tetrahydrofuran) decreased hydrolysis. Lovastatin was selectively hydrolyzed even in the presence of an excess of simvastatin, another antihypercholesterolemic agent that is structurally very similar to lovastatin. This lovastatin 8'-(α -methylbutyryloxy) esterase can be used to prepare a core intermediate for the generation of novel antihypercholesterolemic agents or to purify simvastatin prepared by C methylation of the 2(S)-methylbutyryloxy side chain of lovastatin.

Lovastatin and simvastatin are structurally related compounds that are clinically useful as potent antihypercholesterolemic agents (2, 25, 29). Cholesterol-lowering therapy with a compound such as lovastatin or simvastatin reduces serum cholesterol levels and slows the progression of atherosclerosis (5, 21, 31, 32). Both lovastatin and simvastatin are competitive and reversible inhibitors of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), which catalyzes the NADPH-dependent reduction of 3-hydroxy-3-methylglutaryl-CoA to mevalonate. This conversion is an early and rate-limiting step in cellular cholesterol biosynthesis. Lovastatin, also known as mevinolin and monacolin K, is produced as a secondary metabolite by fungi such as *Aspergillus terreus* (3), *Monascus ruber* (12), *Pleurotus ostreatus* (15), and some species of *Penicillium* (1). Related compounds, such as compactin (6-demethylmevinolin; ML 236B), have been isolated from *Penicillium citrinum* and *Penicillium brevicompactum* (1, 11). Simvastatin may be generated by C methylation of the natural 2(S)-methylbutyryloxy side chain of lovastatin, as shown in Fig. 1 (26). Lovastatin and simvastatin differ only in that lovastatin has a 2-methylbutyryloxy side chain at the 8' position while simvastatin has a 2,2-dimethylbutyryloxy side chain.

Three instances exist in which the hydrolysis of lovastatin to its component parts of diol lactone and methylbutyrate (Fig. 1) would be advantageous. In the first case, diol lactone generated by the removal of the methylbutyrate side chain from lovastatin has itself been shown to exhibit some inhibition of HMG-CoA reductase activity (20) and may be useful as a cholesterol-lowering agent. Second, diol lactone is potentially valuable as an intermediate to the creation of other semisynthetic HMG-CoA reductase inhibitors (10, 20). Diol lactone could provide a core molecule to which a variety of modifications could be made to generate different and possibly novel HMG-CoA reductase inhibitors. Third, the preparation of simvastatin by direct methylation of lovastatin as described by

Sleteinger et al. (26) is hampered by the presence of residual, unconverted lovastatin. Lovastatin and simvastatin are structurally similar and difficult to separate. However, lovastatin selectively hydrolyzed to diol lactone can be readily separated from simvastatin (by crystallization, as an example). Thus, an esterase capable of selective and quantitative cleavage of the methylbutyrate side chain from lovastatin would be useful in generating diol lactone for use as an HMG-CoA reductase agent, in the search for novel HMG-CoA reductase inhibitors, and in removing residual lovastatin during the preparation of simvastatin by direct methylation of lovastatin.

The occurrence in nature of such an esterase was described in a report by Komagata et al. (20) where the hydrolysis of lovastatin by dried mycelia of the fungus *Emericella unguis* was shown. Unfortunately, the highest level of lovastatin hydrolysis obtained by these investigators (with a substrate concentration of 1 mM) was 86% of the starting substrate. Additionally, the activity reported dropped rapidly at substrate concentrations above 5 mM. Ideally, a robust and potentially useful lovastatin esterase would remain active at much higher substrate concentrations. For that reason, a search was conducted to examine other microbial strains for an esterase that could selectively cleave methylbutyrate from lovastatin. Especially desirable would be an esterase that would selectively cleave lovastatin in the presence of simvastatin. Cultures tested by Komagata et al. (20) as well as random cultures available in the Merck culture collection were screened for such an esterase. Over 150 microorganisms, representing equal numbers of bacteria (including actinomycetes) and fungi, were screened. Of these, the fungus *Clonostachys compactiuscula* produced an enzyme which was shown to be capable of complete hydrolysis of lovastatin ammonium salt (LAS) to its component parts, triol (open form of diol lactone) and methylbutyric acid, and to do so even in the presence of simvastatin concentrations which are several times that of lovastatin. This paper describes the purification, properties, and utility of this lovastatin 8'-(α -methylbutyryloxy) esterase from *C. compactiuscula*.

MATERIALS AND METHODS

Materials. Glucose, buffers, and other reagents were obtained from Sigma Chemical Co. (St. Louis, Mo.). With the exception of corn steep liquor, which

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FIG. 1. (A) Formation of simvastatin by methylation of 2-methylbutyryloxy side chain of lovastatin; (B) formation of diol lactone and methylbutyrate from lovastatin by the action of *C. compactusculosa* lovastatin esterase.

was obtained from Grain Processing Corp. (Muscatine, Iowa), culture medium ingredients (yeast extract, malt extract, beef extract, and peptone) were obtained from Difco (Detroit, Mich.). Solvents used were obtained from Fisher Scientific (Pittsburgh, Pa.). Lovastatin, diol lactone, and simvastatin as ammonium salts and simvastatin prepared by direct methylation of lovastatin were obtained from Greg Tewalt (Chemistry Section, Merck and Co., Elkton, Va.).

Growth and fermentation conditions. *C. compactusculosa* ATCC 38009 was obtained from the Merck Culture Collection (Rahway, N.J.). The culture was maintained on yeast extract-malt extract (YME) agar slants containing 0.4% yeast extract, 1% malt extract, 0.4% glucose, and 2% agar (pH 7.0). Freshly inoculated slants were incubated at 28°C for 5 days, after which they were stored at 4°C. The culture growth medium used throughout was that described by Komagata et al. (20) and consisted of glucose (1.0%), peptone (0.2%), beef extract (0.1%), yeast extract (0.1%), and corn steep liquor (0.3%; pH 7.2). Just prior to use, each YME agar slant was washed with 5 ml of sterile water to produce an inoculum with approximately 10^6 spores/ml. Individual 250-ml Erlenmeyer flasks were inoculated with 1 ml of this slant wash in 20 ml of culture growth medium. These flasks were incubated at 27°C on a rotary shaker at 220 rpm. LAS was added aseptically at day 3 (determined empirically to be the best time for enzyme induction) to a concentration of 2 mg/ml, and the culture was allowed to incubate for another 2 days, at which time it was harvested and the enzyme was purified as described below.

Purification. *C. compactusculosa* mycelia from 5-day-old fermentations were harvested by centrifugation ($1,000 \times g$ for 10 min) and washed once with 50 mM Tris (pH 7.7). The mycelia were then homogenized by mixing approximately 20 g of fungal tissue with ground glass and dry ice in a prechilled mortar and grinding with a ceramic pestle. The frozen homogenate was allowed to thaw and was then centrifuged at $1,000 \times g$ for 10 min at 4°C to pellet whole cells and cell debris. All subsequent steps were performed at 4°C. The $1,000 \times g$ supernatant was centrifuged at $30,000 \times g$ to obtain a clear, straw-colored cell extract. The cell extract was filtered through a 0.45- μ m-pore-size filter and purified with a Mono Q ion-exchange column (Pharmacia, Piscataway, N.J.) with 20 mM Tris (pH 8.5) as the mobile phase (buffer A). The esterase was eluted from the column with a linear gradient of sodium chloride (0 to 500 mM in 20 mM Tris [pH 8.5]) at a flow rate of 4 ml/min.

Esterase enzyme assay. Standard conditions for the lovastatin esterase assay consisted of glycine buffer (100 mM, pH 9.4), 10% methanol, 10 mM lovastatin ammonium salt, 4.0 μ g of purified enzyme, and water to a final volume of 1 ml. The mixture was incubated for 15 min at 28°C. Enzyme activity in *C. compactusculosa* culture broth was determined as described above with the substitution of a known volume of whole broth for the purified enzyme. Any other variation to these standard conditions is described in the individual experiment. The conversion of lovastatin to triol (open form of diol lactone) was quantified by isocratic reverse-phase chromatography (HPLC) with a Pecosphere octyldecyl silane column with a 3.3-cm column length and 3- μ m particle size (Perkin-Elmer, Norwalk, Conn.). The mobile phase of 0.1% aqueous phosphoric acid and acetonitrile

TABLE 1. Induction of *C. compactusculosa* lovastatin esterase by lovastatin-related compounds

Inducer	Concn added (g/liter) ^a	Esterase produced ^b	
		U/g of DCW	U/liter of broth
LAS	0.5	20.0	435
	2.0	46.7	845
Simvastatin ammonium salt	0.5	12.5	286
	2.0	22.8	527
Triol ammonium salt	0.5	2.6	55
	2.0	6.6	158
Methyl butyrate	0.5	0	0
	2.0	0	0
	5.0	0	0

^a Each inducer listed was added aseptically to *C. compactusculosa* cultures to the final concentration shown as described in Materials and Methods.

^b Esterase activity was determined, in duplicate, as units of activity per gram of mycelial dry cell weight (DCW) and as units per volume of *C. compactusculosa* culture broth, where a unit is defined as the amount of enzyme required to hydrolyze 1 μ mol of LAS per min.

trile (50/50) was kept constant at a flow rate of 3 ml/min. Detection was accomplished at 238 nm.

Estimation of molecular weight. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to check the protein purity and determine the molecular mass of the purified enzyme under denaturing conditions with a 10% acrylamide gel (22). The proteins were stained with Coomassie brilliant blue R-250, and the molecular weight was estimated based on the concurrently electrophoresed protein standards (Bio-Rad, Hercules, Calif.). In addition, ion-exchange chromatography-purified enzyme was fractionated on a fast protein liquid chromatography (FPLC) Superose 12 gel filtration column (Pharmacia), previously equilibrated with 100 mM Tris (pH 7.8) and 150 mM sodium chloride, at a flow rate of 0.35 ml/min. The elution of the purified lovastatin esterase was compared with that of protein standards (17, 50, 150, and 465 kDa; Boehringer Mannheim, Indianapolis, Ind.) assayed under conditions identical to those of the purified enzyme to determine the molecular weight of the native protein.

Protein assays. Protein concentrations were determined by the Bradford method (6) with Coomassie protein assay reagent (Pierce, Rockford, Ill.) and bovine serum albumin standards.

Dry cell weight determination. The dry cell weight was determined by filtering the culture broth through filter paper (Whatman 934-AH; Fisher Scientific) and drying the remaining mycelial mat overnight at 60°C.

RESULTS

Induction of the lovastatin esterase. The lovastatin esterase activity in *C. compactusculosa* was originally discovered in an activity screen that used LAS as a substrate which was subsequently shown to be effective as an inducer of the esterase activity. The addition of lovastatin or lovastatin-related compounds (simvastatin or diol lactone) to the *C. compactusculosa* culture medium was absolutely required to induce the expression of the lovastatin esterase. Lovastatin, as LAS, was most effective in inducing the esterase from *C. compactusculosa*. Simvastatin ammonium salt and triol ammonium salt (salt of diol lactone) were less effective but still induced the expression of moderate levels of lovastatin esterase. One product of lovastatin hydrolysis by the lovastatin esterase, methyl butyrate, was ineffective in inducing enzyme expression when added to cultures of *C. compactusculosa*. These results are shown in Table 1. A number of other esters (ethyl butyrate, ethyl acetoacetate, methyl acetoacetate, ethyl acetate, isobutyric acid methyl ester, ethyl malonic acid dimethyl ester, ethyl sodium oxaloacetate, glutaric acid dimethyl ester, and methyl α -D-glucoside) were tested and found totally ineffective in inducing esterase activity (data not shown).

Purification. Homogenization of 5-day-old mycelial pellets (induced with LAS on day 3) was carried out as detailed in Materials and Methods. The soluble lovastatin esterase in the

FIG. 2. SDS-PAGE of lovastatin esterase-containing fractions. A 10% acrylamide gel of esterase-containing fractions at different stages of purification is shown. Lanes: 1, post-1,000 $\times$ g supernatant; 2, post-30,000 $\times$ g supernatant; 3, ion-exchange chromatography-purified lovastatin esterase. The migration of the molecular mass markers is shown by the bars to the right of the gel.

30,000 $\times$ g cell extract was purified by anion-exchange chromatography on an FPLC Mono Q HR 10/10 column. The esterase bound to the anion-exchange resin at pH 7.8 and eluted at 275 mM sodium chloride. The lovastatin esterase was readily separated from the majority of *C. compactiuscula* proteins by this procedure, with the purified protein showing a single band of approximately 45,000 Da by SDS-PAGE (Fig. 2). Esterase purified in this manner retained 100% activity after more than 3 months whether stored at 4 or -80°C . The enzyme purified by anion-exchange chromatography was used for all studies described below.

Determination of optimal conditions. The pH range for maximal esterase activity was tested with 100 mM glycine buffer at pH intervals of 0.2 from pH 8.4 to 10.4. The greatest esterase activity occurred at pH 9.4, but there was little difference in the rate of hydrolysis in the samples at pH 9.0 to 9.6 (Fig. 3A). A pH of 9.4 was chosen as the standard condition and used to test the optimal temperature for enzymatic activity. LAS hydrolysis was monitored at 15, 20, 25, 28, 30, 35, 40, and 45°C over a 6-h period. The results, shown in Fig. 3B, indicate that optimal enzyme activity occurs between 25 and 30°C . A temperature of 28°C was chosen for the standard conditions.

Solvents have been shown to enhance the action of a number of enzymes (18). This phenomenon is attributed to the optimization of conformational changes in the enzyme induced by the organic solvent or the solvent acting as an additional nucleophile. In addition, the inclusion of particular solvents may act to simply increase the solubility of the reaction substrate if it is not soluble in water. The addition of ethyl acetate, isopropyl acetate, tetrahydrofuran, and methanol to the enzyme mixture at a solvent concentration of 5, 10, 15, and 20% was evaluated. Methanol was the only solvent that resulted in a greater degree of LAS hydrolysis than that of the control reaction with no organic solvent present. Due to the increased hydrolysis when methanol was present, the effect of C_1 to C_4

FIG. 3. pH (A) and temperature (B) optima of the lovastatin esterase. Enzymatic activity was determined as described in Materials and Methods for the times indicated.

alcohols on esterase activity was investigated. Methanol was tested again as well as ethanol, isopropyl alcohol, and butanol. Each solvent was evaluated at 5, 10, 15, and 20% final concentrations. Again, of these solvents, only methanol had a positive effect on the degree of LAS hydrolysis as compared to that of water alone. There was a mean increase in lovastatin hydrolysis of 12.0% (standard error of the mean, 1.5%) for five separate experiments when methanol was used at a final concentration of 10%. The amount of hydrolysis decreased with increasing chain lengths of the alcohols (i.e., methanol > ethanol > isopropanol > butanol).

Physical and kinetic properties. The molecular mass of the denatured enzyme was estimated to be 45 kDa by SDS-PAGE after the purified enzyme had been boiled in the presence of SDS and 2-mercaptoethanol (Fig. 2). The molecular mass of the native enzyme was calculated to be 46.0 kDa by Superose 12 gel filtration (as described in Materials and Methods) as compared to protein standards separated under identical conditions. The agreement of the calculated molecular mass by both methods suggests that the enzyme exists as a monomer, as found in the case of other recently reported fungal esterases (13, 23). The effect of substrate concentration on the reaction rate was assayed by using the standard enzyme assay conditions. The Michaelis constant (K_m) and maximal velocity for the reaction with lovastatin as the substrate were calculated from Lineweaver-Burk plots. The K_m value for lovastatin was 0.35 mM, and the maximal velocity was 16.1 $\mu\text{mol}/\text{min}$ per mg of protein.

FIG. 4. Hydrolysis of lovastatin (as lovastatin ammonium salt) by *C. compactuscula* lovastatin esterase in the presence of simvastatin. Lovastatin remaining after incubation with esterase was assayed by HPLC as described in Materials and Methods. The initial concentration of LAS was 10 mM, while the simvastatin ammonium salt concentration varied as indicated in the figure.

Hydrolysis of lovastatin in the presence of simvastatin. An esterase which could cleave lovastatin to diol lactone in the presence of simvastatin would be useful in the separation of simvastatin from unreacted lovastatin during the production of simvastatin by C methylation of the 2-methylbutyryloxy side chain of lovastatin. Lovastatin is difficult to separate from the newly formed simvastatin, which has a 2,2-dimethyl-butyl side chain at the 8' position. Selective cleavage of the 2-methylbutyryloxy side chain from any unreacted lovastatin by the action of the lovastatin esterase would yield diol lactone, which can be readily separated by crystallization from the simvastatin produced. The hydrolysis of a constant 10 mM lovastatin was tested in the presence of increasing amounts of simvastatin by using the standard conditions of pH 9.4 and 28°C with 10% methanol and 4.0 µg of purified enzyme. These results are presented in Fig. 4. In the absence of any simvastatin, the lovastatin was 80% hydrolyzed in less than 2 h. With increasing levels of simvastatin, it took longer to accomplish the same degree of hydrolysis. Yet, even with threefold-greater simvastatin than lovastatin, the lovastatin was 80% hydrolyzed in 4 h. Simvastatin alone was not hydrolyzed to any great extent. When 10 mM simvastatin ammonium salt was incubated with enzyme under conditions identical to those described above for lovastatin, 97.5% of the simvastatin remained unhydrolyzed even after 20 h.

DISCUSSION

This work had as its goal the search and characterization of a hydrolase capable of specifically cleaving an ester bond in lovastatin to yield diol lactone and methylbutyrate. Such an esterase was found in and purified from the fungus *C. compactuscula*. The lovastatin esterase from *C. compactuscula* was not constitutively produced but required the addition of lovastatin (or lovastatin-related compounds) for expression. Other ester-containing compounds that were tested that did not contain the diol lactone portion of lovastatin did not induce the esterase activity. It is interesting that even diol lactone alone was sufficient to induce the lovastatin esterase (albeit at a lower level), even though the ester bond that is cleaved by the induced esterase is missing in diol lactone. Also, simvastatin, while not actively cleaved by the esterase, was active in inducing the esterase. The addition of methanol resulted in a slight but consistent increase in total amount of lovastatin hydrolyzed. This increase may be due to optimization of conforma-

tional changes in the enzyme but is just as likely to be due to an increase in the solubility of lovastatin with the addition of a small amount of methanol, since lovastatin has only limited solubility in water alone.

In addition to the HMG-CoA reductase inhibitors that occur in nature, such as lovastatin produced by *Aspergillus terreus* (3) and compactin produced by *Penicillium citrinum* (11), a number of semisynthetic analogs have been generated and shown to possess HMG-CoA reductase inhibitory activity (16, 27). In some cases, these semisynthetic analogs have enhanced activity as compared to the naturally produced HMG-CoA reductase inhibitors. Several of these semisynthetic HMG-CoA reductase inhibitors retain a similar diol lactone core structure. In the case of simvastatin, a 2,2-dimethylbutyryl side chain replaces the 2-methylbutyryl side chain at carbon-1 of lovastatin. A useful starting molecule in the synthesis of novel analogs, therefore, is the deacylated diol lactone. In this manner, diol lactone can be likened to 6-aminopenicillanic acid (6-APA), which is used as an intermediate in the synthesis of many semisynthetic penicillin antibiotics. Penicillin acylases produced by *Escherichia coli*, *Bacillus* sp., *Nocardia* sp., or *Alcaligenes faecalis* will cleave penicillin G that is produced by *Penicillium chrysogenum* and *Aspergillus nidulans* (7, 17, 28). These acylases hydrolyze the side chain from penicillin G to produce 6-APA, which, in turn, is used to chemically derive a wide variety of semisynthetic penicillin analogs. New 6-APA derivatives continue to be discovered even though the enzymatic hydrolysis of penicillin G to 6-APA was reported over 30 years ago (9). Recent reports in the literature describe *tert*-butyl esters of 6-APA (14), enzymatic synthesis of pivampicillin with 6-APA (19), preparation of penicillin sulfones from 6-APA (30), and the formation of 8-hydroxypenicillin from 6-APA (8). The preparation and use of 6-APA as a core molecule to which modifications can be made allow not only the synthesis of a large number of novel penicillin derivatives but also have aided in the elucidation of the biosynthesis for several natural penicillins (24). Potentially, diol lactone may serve an equivalent purpose in the discovery of novel semisynthetic HMG-CoA reductase inhibitors. The use of core molecules such as 6-APA or diol lactone as intermediates accelerates drug discovery and allow molecules to be tailored to increase bioavailability or lessen toxicity.

A method to convert lovastatin to simvastatin has been reported but is complicated by the difficulty in removing residual lovastatin (4, 26). Repeated crystallizations of a mixture of trace lovastatin in simvastatin results only in the enrichment of lovastatin (4). The selectivity of this esterase is fortuitous for the hydrolysis of lovastatin in a mixture of simvastatin. The lovastatin esterase from *C. compactuscula* provides a means to isolate simvastatin from mixtures of simvastatin and lovastatin since it does not readily cleave simvastatin but actively hydrolyzes lovastatin (even in the presence of an excess amount of simvastatin). This lovastatin 8'-(α -methylbutyryloxy) esterase from *C. compactuscula* is useful in generating diol lactone for use as an intermediate to novel HMG-CoA reductase inhibitors and in removing residual starting material from simvastatin preparations made by the methylation of lovastatin.

REFERENCES

1. Alberts, A. W. 1988. Discovery, biochemistry and biology of lovastatin. *Am. J. Cardiol.* 62:10J-15J.
2. Alberts, A. W. 1990. Effect of HMG CoA reductase inhibitors on cholesterol synthesis. *Drug Invest.* 2:9-12.
3. Alberts, A. W., J. Kuron, V. Hunt, J. Huff, J. Hoffman, J. Rothrock, M. Lopez, H. Joshua, E. Harris, A. Patchett, R. Monaghan, S. Currie, E. Stapley, G. Albers-Schonberg, O. Hensens, J. Hirshfield, K. Hoogsteen, J. Liesch, and J. Springer. 1980. Mevinolin: a highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-low-

- ering agent. Proc. Natl. Acad. Sci. USA **77**:3957–3961.
4. Askin, D., T. R. Verhoeven, T. M.-H. Liu, and I. Shinkai. 1991. Synthesis of synvinolin: extremely high conversion alkylation of an ester enolate. J. Org. Chem. **56**:4929–4932.
 5. Blankenhorn, D. H., S. P. Azen, D. M. Kramsch, W. J. Mack, L. Cashin-Hemphill, H. N. Hodis, L. W. V. DeBoer, P. R. Mahrer, M. J. Masteller, L. I. Vailas, P. Alaupovic, L. J. Hirsch, and the MARS Research Group. 1993. Coronary angiographic changes with lovastatin therapy. Ann. Intern. Med. **119**:969–976.
 6. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
 7. Burnett, W. V., and T. G. Heckler. 1989. Industrial enzymes in drug development, p. 133–154. In J. M. Tabor (ed.), Genetic engineering technology in industrial pharmacy. Marcel Dekker, Inc., New York, N.Y.
 8. Casy, A. F., and A. Lipczynski. 1994. 8-Hydroxyphenillic acid—NMR characteristics and facile formation from 6-aminopenicillanic acid. J. Pharm. Pharmacol. **46**:533–534.
 9. Claridge, C. A., A. Gourevitch, and J. Lein. 1960. Formation of 6-aminopenicillanic acid from penicillin by enzymatic hydrolysis. Nature **187**:236–238.
 10. Cover, W. H., R. L. Dabora, A. Hong, C. Reeves, R. W. Stieber, and V. A. Vinci. October 1993. Mutant strains of *Aspergillus terreus* for producing 7-[1,2,6,7,8,8A(R)-hexa-hydro-2(S), 6(R)-dimethyl-8(S)-hydroxy-1(S)-naphthyl]-3(R)-dihydroxyheptanoic acid (triol acid). U.S. patent 5250435.
 11. Endo, A., M. Kuroda, and Y. Tsujita. 1976. ML-236A, ML-236B, and ML-236C, new inhibitors of cholesterologenesis produced by *Penicillium citrinum*. J. Antibiot. **29**:1346–1348.
 12. Endo, A. 1979. Monacolin K, a new hypocholesterolemic agent produced by a *Monsacus* species. J. Antibiot. **32**:852–854.
 13. Faulds, C. B., and G. Williamson. 1993. Ferulic acid esterase from *Aspergillus niger*—purification and partial characterization of two forms from a commercial source of pectinase. Biotechnol. Appl. Biochem. **17**:349–359.
 14. Grigan, N., D. Musel, G. A. Veinberg, and E. Lukevics. 1996. A simple preparative method for tert-butyl protection of aminocephalosporanic acid. Synth. Commun. **26**:1183–1185.
 15. Gunde-Cimerman, N., A. Plenenitas, and A. Cimerman. 1993. Pleurotus fungi produce mevinolin, an inhibitor of HMG-CoA reductase. FEMS Microbiol. Lett. **113**:333–337.
 16. Hoffman, W. F., A. W. Alberts, P. S. Anderson, J. S. Chen, R. L. Smith, and A. K. Willard. 1986. 3-Hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors. 4. Side chain ester derivatives of mevinolin. J. Med. Chem. **29**:849–852.
 17. Illanes, A., F. Acevedo, J. C. Gentina, I. Reyes, R. Torres, O. Caragena, A. Ruiz, and M. Vasquez. 1994. Production of penicillin acylase from *Bacillus megaterium* in complex and defined media. Process Biochem. **29**:263–270.
 18. Khmelnitsky, Y. L., A. V. Levashov, N. L. Klyachko, and K. Martinek. 1988. Engineering biocatalytic systems in organic media with low water content. Enzyme Microb. Technol. **10**:710–715.
 19. Kim, M. G., and S. B. Lee. 1996. Penicillin acylase-catalyzed synthesis of pivampicillin—effect of reaction variables and organic cosolvents. J. Mol. Catal. B Enzymatic **1**:71–80.
 20. Komagata, D., H. Yamashita, and A. Endo. 1986. Microbial conversion of compactin (ML-236B) to ML-236A. J. Antibiot. **39**:1574–1577.
 21. Krauss, R. M. 1994. Heterogeneity of plasma low-density lipoproteins and atherosclerosis risk. Curr. Opin. Lipidol. **5**:339–349.
 22. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227**:680–685.
 23. Linden, J., M. Samara, S. Decker, E. Johnson, M. Boyer, M. Pecs, W. Adney, and M. Himmel. 1994. Purification and characterization of an acetyl esterase from *Aspergillus niger*. Appl. Biochem. Biotechnol. **45–46**:383–93.
 24. Luengo, J. M. 1995. Enzymatic synthesis of hydrophobic penicillins. J. Antibiot. **48**:1195–1212.
 25. Plosker, G. L., and D. McTavish. 1995. Simvastatin: a reappraisal of its pharmacology and therapeutic efficacy in hypercholesterolaemia. Drugs **50**:334–363.
 26. Sleteinger, M., T. Verhoeven, and R. Volante. April 1986. One step process for C-methylation of 2-methylbutyrates. U.S. patent 4,582,915.
 27. Stokker, G. E., A. W. Alberts, J. L. Gilfillan, J. W. Huff, and R. L. Smith. 1986. 3-Hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors. 5. 6-(Fluoren-9-yl)- and 6-(fluoren-9-ylidenyl)-3,5-dihydroxyhexanoic acids and their lactone derivatives. J. Med. Chem. **29**:852–855.
 28. Sudhakaran, V. K., B. S. Deshpande, S. S. Ambedkar, and J. G. Shewale. 1992. Molecular aspects of penicillin and cephalosporin acylases. Process Biochem. **27**:131–143.
 29. Tobert, J. A. 1987. New developments in lipid-lowering therapy: the role of inhibitors of hydroxymethylglutaryl-coenzyme A reductase. Circulation **76**:534–538.
 30. VanWetswinkel, S., J. Fastrez, and J. Marchandbrynaert. 1994. Synthesis of new sulfonylamido-penicillanic acid sulfones. J. Antibiot. **47**:1041–1051.
 31. Walker, J. F., and J. A. Tobert. 1988. The clinical efficacy and safety of lovastatin and MK-733—an overview. Eur. Heart J. **8**(Suppl. E):93–96.
 32. Waters, D., L. Higginson, P. Gladstone, B. Kimball, M. LeMay, S. J. Bocuzzi, J. Lesperance, and the CCAIT Study Group. 1994. Effects of monotherapy with an HMG-CoA reductase inhibitor on the progression of coronary atherosclerosis as assessed by serial quantitative arteriography—the Canadian coronary atherosclerosis intervention trial. Circulation **89**:959–968.