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Biochemistry

Mevinolin: A highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent

[fungal metabolites/crystal structure/absolute configuration/cholesterol synthesis/ML-236B (compactin)]

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ABSTRACT **Mevinolin, a fungal metabolite, was isolated from cultures of *Aspergillus terreus*. The structure and absolute configuration of mevinolin and its open acid form, mevinolinic acid, were determined by a combination of physical techniques. Mevinolin was shown to be 1,2,6,7,8,8a-hexahydro-β,δ-dihydroxy-2,6-dimethyl-8-(2-methyl-1-oxobutoxy)-1-naphthaleneheptanoic acid δ-lactone. Mevinolin in the hydroxy acid form, mevinolinic acid, is a potent competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase [mevalonate: NADP$^+$ oxidoreductase (CoA-acylating), EC 1.1.1.34]; its K_i of 0.6 nM can be compared to 1.4 nM for the hydroxy acid form of the previously described related inhibitor, ML-236B (compactin, 6-demethylmevinolin). In the rat, orally administered sodium mevinolinate was an active inhibitor of cholesterol synthesis in an acute assay (50% inhibitory dose = 46 μg/kg). Furthermore, it was shown that mevinolin was an orally active cholesterol-lowering agent in the dog. Treatment of dogs for 3 weeks with mevinolin at 8 mg/kg per day resulted in a 29.3 ± 2.5% lowering of plasma cholesterol.**

The major cause of death in the western countries is coronary artery disease. A primary risk factor for the disease is known to be hypercholesterolemia (1, 2). In humans 50% or more of the total body cholesterol is derived from *de novo* synthesis (3). A major rate-limiting step in the cholesterol biosynthetic pathway is at the level of the microsomal enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase [HMG-CoA reductase; mevalonate:NADP$^+$ oxidoreductase (CoA-acylating), EC 1.1.1.34] (4). This enzyme, therefore, is a prime target for pharmacological intervention.

Recently, Endo and his associates isolated and characterized a metabolite from cultures of *Penicillium citrinum* that was shown to be an extremely potent competitive inhibitor of HMG-CoA reductase (5–8). This compound, designated ML-236B, is identical to one isolated from *P. brevicompactum* as an antifungal agent, which was named compactin (9). ML-236B was shown to be a potent inhibitor of cholesterol synthesis from acetate but not from mevalonate in a number of cell types in culture (7, 10–13) as well as in acutely treated rats (14). Of great significance was the observation that, although it did not affect plasma cholesterol levels in chronically treated rats (11, 15) or mice (15), ML-236B effectively reduced plasma cholesterol levels in a number of other species including dogs (16), monkeys (17) and humans (18).

In this communication we describe the isolation, structure, and biochemical properties of a more active inhibitor of HMG-CoA reductase, mevinolin (Fig. 1, structure **1**), and its corresponding open acid form, mevinolinic acid (Fig. 1, structure **2**) obtained from cultures of a strain of *aspergillus terreus*.

MATERIALS AND METHODS

Cultures and Media. A strain of *A. terreus*, ATCC 20542, obtained from a soil isolation program at the CEPA Laboratories (Madrid, Spain) was used to produce mevinolin. The culture was maintained at 28°C on agar slants composed of, per liter: 4 g of yeast extract, 10 g of malt extract, 4 g of dextrose, and 20 g of agar (at pH 7.0).

Mevinolin was produced in a multi-stage fermentation process consisting of cell propagation in medium A and product formation in medium B. Medium A contained, per liter: 5 g of corn steep liquor, 40 g of tomato paste, 10 g of oat flour, 10 g of dextrose, and 10 ml of a trace element solution, pH 6.8. The trace element solution was composed of, per liter: 1 g of $FeSO_4 \cdot 7H_2O$, 1 g of $MnSO_4 \cdot 4H_2O$, 25 mg of $CuCl_2 \cdot 2H_2O$, 100 mg of $CaCl_2 \cdot 2H_2O$, 56 mg of H_3BO_3, 19 mg of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, and 200 mg of $ZnSO_4 \cdot 7H_2O$. Medium B contained, per liter: 45 g of dextrose, 24 g of peptonized milk, 2.5 g of yeast extract, 2.5 ml of polyethylene glycol P2000 (at pH 7.4). Mevinolin has been produced successfully in flasks and fermentors. Maximal production occurred, with agitation, during 4–6 days of incubation at 28°C in medium B after inoculation (5–10%) with the growth in medium A.

Isolation and Characterization of Mevinolin. A 200-gallon (1 gallon = 3.79 liters) culture of *A. terreus* incubated for 6 days in medium B as described above was combined with 60 gallons of water, acidified to pH 4.2, and stirred with 75 gallons of ethyl acetate for 2 hr; then 50 pounds (23 kg) of Supercel filter aid was added and the slurry was filtered through a filter press. The press cake was washed with an additional 75 gallons of ethyl acetate. The combined extracts were washed once with 15 gallons of water, separated, dried over 2 kg of magnesium sulfate, filtered, and concentrated under reduced pressure. The concentrate was stripped of the residual solvent on a rotary vacuum evaporator and flushed with an equal volume of toluene and reconcentrated. To complete the lactonization of mevinolinic acid to mevinolin, 8 liters of toluene was added and the batch was refluxed for 2 hr while a small amount of water and solvent was distilled off at atmospheric pressure.

The toluene was removed by evaporation under reduced pressure and the residue was flushed once by addition of an equal volume of methylene chloride and reconcentrated to a

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Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; ML-236B, compactin (6-demethylmevinolin).

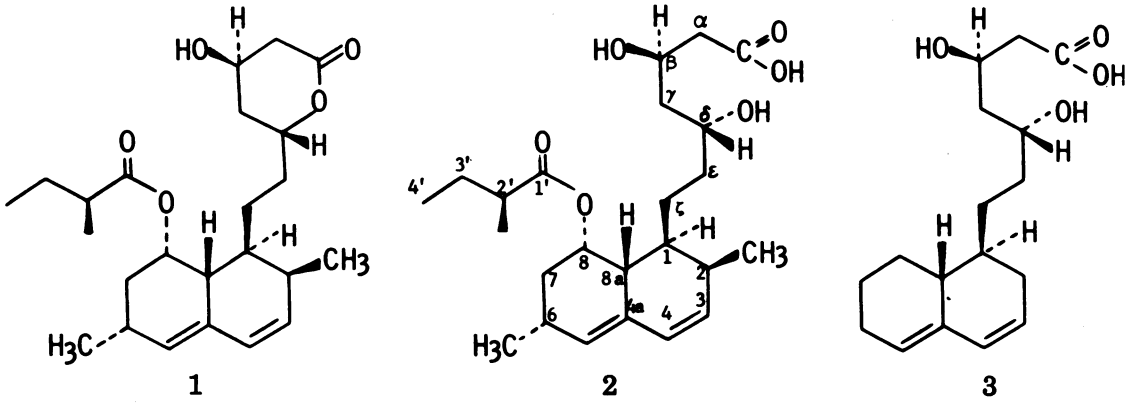

FIG. 1. Structures of mevinolin (1), mevinolinic acid (2), and mevinic acid (3), showing the correct absolute configuration.

dark oily residue, which was dissolved in 8 liters of ethyl acetate/methylene chloride (30:70, vol/vol) and made into a slurry with 1.3 kg of silica gel. This was charged onto a 23-cm (inside diameter) glass column packed with 15 kg of silica gel in the same solvent. Elution with ethyl acetate/methylene chloride (40:60, vol/vol) was begun at a flow rate of 300 ml/min. After the initial 5 gallons had been discarded, 40 fractions of 1 gallon each were collected. The loading zone of the column was stirred occasionally during the early development to promote flow through the tarry aggregate. A high content of mevinolin was found by high-pressure liquid chromatography assay in fractions 20–34, which were combined and concentrated under reduced pressure to a partly solid residue.

The residue was taken up in 1.5 liter of hot ethyl acetate. Cooling, filtration, and drying yielded 111 g of nearly white crystals of mevinolin. Two further recrystallizations from acetonitrile, and then ethyl acetate and drying 16 hr at 40°C under reduced pressure, yielded 79 g of pure mevinolin, $[\alpha]_D^{25}$ = +323° (0.5 g in 100 ml of CH_3CN); UV λ_{max} 247, 238, and 231 nm, $A^{1\%}$ 418, 621, and 532; differential thermal analysis, mp (under N_2) 174.5°C.

High-resolution mass spectra were obtained on a Varian MAT-731 spectrometer. ^1H and ^{13}C NMR spectra were obtained at 300 MHz and 75 MHz, respectively, using a Varian SC-300 NMR spectrometer in the Fourier transform mode. ^1H NMR spectra were recorded at 50°C in C^2HCl_3 spiked with ^2H_2O; chemical shifts δ are given in ppm relative to internal tetramethylsilane. ^{13}C NMR spectra were recorded in C^2HCl_3 at 25°C.

Isolation and Assay of HMG-CoA Reductase. Microsomes were prepared from livers of rats that had been maintained on rat chow containing cholestyramine for 7 days. HMG-CoA reductase was solubilized from the microsomes by the method of Heller and Shrewsbery (19) and purified through the second ammonium sulfate precipitation step as described by Kleinsek *et al.* (20). The enzyme preparation was stored at −80°C in 100-μl samples and was stable for at least 3 months in this form. Prior to use, the enzyme was activated at 37°C for 30 min. The assay was similar to that described by Beg *et al.* (21). Except where indicated, the reaction mix contained, in 100 μl: 0.14 M potassium phosphate buffer, pH 6.8; 0.18 M KCl; 3.5 mM EDTA, pH 7.0; 10 mM dithiothreitol; bovine serum albumin at 0.1 mg/ml; 0.02 μCi of [^{14}C]HMG-CoA (New England Nuclear; 1 Ci = 3.7 × 10^{10} becquerels) at the concentration indicated in the text; and 0.3 μg of partially purified enzyme (specific activity 100–150 nmol min^{-1} mg^{-1}) with or without inhibitor. After 5-min incubation at 37°C, the reaction was initiated with 0.2 mM NADPH. The reaction was terminated with 20 μl of 5 M HCl. After an additional incubation for 15 min at 37°C to allow for complete lactonization of the product, mevalonate, the mixture was passed over a 0.5 × 5 cm column containing 100–200 mesh Bio-Rex, chloride form (Bio-Rad), which was equilibrated with distilled water. With this resin the unreacted [^{14}C]HMG-CoA was adsorbed and the product, [^{14}C]mevalonolactone, was eluted with 3 ml of distilled water directly into scintillation vials. After the addition of 10 ml of Aquasol II (New England Nuclear), radioactivities of the samples were measured in a Packard Model B2450 scintillation counter.

Mevinolin and ML-236B were converted into their respective sodium salts as described (6). Prior to assay the inhibitors were appropriately diluted with dimethyl sulfoxide. Dilution to concentrations below 0.1 μM with aqueous buffers resulted in erratic results, which were attributed to partial inactivation of the inhibitors under these conditions.

Acute Inhibition of Cholesterol Synthesis in Rats. Holtzman male albino rats were obtained from the supplier at 100–110 g and maintained on Purina Formulab Chow-5008 for 1 week. On the seventh day, the rats were divided into seven or nine groups of 10 animals per group of equal average weight (approximately 160 g) and feeding ad lib on the Formulab Chow was continued. The animals were housed 10 per cage in wire-bottom cages in air-conditioned quarters. At 8:30 a.m. on the following day, the food was removed from the cages and each group of rats was given a single stomach tube dose of the test compound suspended in 5% Emulphor in saline at the doses indicated in the figures. The control group received the suspending vehicle only. One hour after compound administration, the rats were injected intraperitoneally with sodium [1-^{14}C]-acetate (26.7 μCi/ml, specific activity 23.1 μCi/mg) at 80 μCi/kg. After 50 min, 4-ml blood samples were taken by cardiac puncture, while the animals were under light pentobarbital anesthesia, and were placed in tubes containing 0.2 ml of 0.4 M sodium citrate. The plasma, obtained by centrifugation, was hydrolyzed and the cholesterol was extracted into petroleum ether according to the method of Abell *et al.* (22). For the measurement of plasma [^{14}C]cholesterol, 2 ml of the petroleum ether solution was evaporated to dryness in 8-ml counting vials with mild heating and taken up in 5 ml of Aquasol-2, and the ^{14}C content was determined with a model PLD Prias liquid scintillation counter.

Chronic Dog Studies. Pure-bred male beagles obtained from Haycock Kennels (Quakertown, PA) were housed individually and fed milled Purina Lab Canine diet at 30 g/kg per day. Prior to the start of the experiment, the dogs were bled twice weekly from the jugular vein and plasma cholesterol levels were determined by the method of Abell *et al.* (22) until values remained stable. To test the effects of mevinolin on plasma cholesterol, the compound was given to the dogs at 8 mg/kg per day. It was administered mixed with their rations, which were

totally consumed. Bleeding was continued twice weekly for an additional 4 weeks and plasma cholesterol was determined.

RESULTS

Structure Determination. Mevinolin has the elemental composition $C_{24}H_{36}O_5$ (parent ion M^+ 404.2563, calc. 404.2563). Mass spectral fragments at mass-to-charge ratio m/e 386 ($C_{24}H_{34}O_4$), 302 ($C_{19}H_{26}O_3$), and 284 ($C_{19}H_{24}O_2$) resulted from the loss of water, a $C_5H_{10}O_2$ moiety, or both. Important fragment ions furthermore appeared at m/e 269 ($C_{18}H_{21}O_2$), 224 ($C_{17}H_{20}$), 197–199 ($C_{15}H_{18\pm1}$), 174 ($C_{13}H_{18}$), 169 ($C_{13}H_{13}$), 159 ($C_{12}H_{15}$; 100%) and 157 ($C_{12}H_{13}$). The hydroxy function permitted formation of a mono(trimethylsilyl) derivative (M^+ 476). Mild alkaline hydrolysis appeared to open a lactone, leading to a dihydroxy carboxylic acid that formed a tris(trimethylsilyl) derivative; the trimethylsilyl ester group of this derivative was characterized by its selective exchange with [$^2H_{18}$]-bis(trimethylsilyl)trifluoroacetamide (M^+ 647). Strong infrared absorbance of mevinolin near 1720 cm^{-1} is in part assigned to this six-membered lactone; salts of the hydrolysis product show diminished absorbance at 1720 cm^{-1} but a strong band near 1555 cm^{-1}. ^1H NMR decoupling experiments are consistent with a β-hydroxy-δ-lactone. The residual infrared absorbance at 1721 cm^{-1} and a strong band at 1190 cm^{-1} suggested an ester group; the mass spectral loss of a $C_5H_{10}O_2$ moiety, which cannot involve the β-hydroxy lactone, could therefore be interpreted as the MacLafferty elimination of a C_5 carboxylic acid. UV absorbance at 231 (ϵ = 21,490 M^{-1} cm^{-1}), 238 (25,090), and 247 nm (16,890) indicated a trisubstituted heteroannular diene chromophore [λ_{max} (calc.) 237 nm]. The elemental composition then requires two rings in addition to the lactone.

Similar structural features have been reported for the *P. citrinum* (5) of *P. brevicompactum* (9) metabolite ML-236B, $C_{23}H_{34}O_5$, which contains a β-hydroxy-δ-lactone, a bicyclic diene chromophore ("dehydrodecalin"), and an α-methylbutyric acid ester functionality, as shown by spectroscopic analysis and x-ray crystallography (9). The reported mass spectral fragmentation pattern of ML-236B closely parallels that of mevinolin. The analogy includes those fragments that retain only the dehydrodecalin portion of the structure (m/e 159 and 145, respectively, formed by elimination of $C_5H_{10}O_2$ and rupture of the C_1C_{ζ} bond) and thus localizes the additional CH_2 element of mevinolin in this area.

Consistent with the high-resolution mass spectroscopy measurements, the ^{13}C NMR spectrum on "gated" decoupling indicated a total of 24 carbons consisting of four methyl, six methylene, and five methine carbons in the high-field region, three methine carbons carrying oxygen substituents, four olefinic signals consistent with a trisubstituted diene, and two ester carbonyls. The high-field region contained a methyl carbon in addition to that expected for ML-236B and substitution of a methine for a methylene carbon. The position of the new methyl substituent at C6 can be determined unambiguously by a ^1H NMR decoupling experiment. Irradiation of H5 at δ 5.52 sharpens the signal for H6 at δ 2.44. H6 in turn is vicinally coupled to the new methyl group at δ 1.07 and homoallylicly to H8a at δ 2.77. Irradiation at the absorption frequency for H8 at δ 5.40 (doublet of triplets, J = 3, 3, and 3 Hz) sharpens the multiplet for the C7 methylene protons at δ 1.94 and collapses the H8a signal to a doublet of triplets (J = 11.5, $\approx$1.5, and $\approx$1.5 Hz), showing that the latter is *trans*-diaxially disposed to H1. H8a in turn is long-range coupled to H5 ($J \approx$ 1.5 Hz) and H6 ($J \approx$ 1.5 Hz). Irradiation at δ 1.94 (H7) sharpens the multiplet assigned to H6 and collapses the H8 absorption to a doublet (J = 3 Hz). A complete analysis of the complex spin pattern involving the protons at C5, C6, C7, and C6 CH_3 was not possible; however, the small values suggested for the couplings involving H6 and H7α and H7β, as well as those with H5 and H8a, do suggest a flattened half-chair conformation of the fused six-membered ring with the methyl group at C6 *cis* to the ester function at C8 as unambiguously demonstrated by x-ray analysis.

Mevinolin ($C_{24}H_{36}O_5$) was crystallized from ethanol as thick, colorless needles. Preliminary x-ray diffraction experiments indicated that the space group was $P2_12_12_1$ with a = 5.974(4) Å, b = 17.337(19) Å, and c = 22.148(19) Å with Z = 4 for a calculated density of 1.17 g/cm^3. Intensity data were collected on a Syntex $P2_1$ diffractometer with graphite monochromated CuKα radiation. The structure was solved by using a multisolution tangent formula approach (23) and refined by using least-squares techniques (24). At the present stage of refinement the R factor is 9.5%.

The absolute configuration of mevinolin was determined by solution of the crystal structure of the amide prepared by opening the lactone with $S(-)\alpha$-methylbenzylamine. Crystals of the amide ($C_{32}H_{47}NO_5$) formed from ether as clear parallelepipeds with the symmetry $P2_1$ and Z = 4. Cell constants were a = 22.340(10) Å, b = 5.701(2) Å, c = 26.453(15) Å, and β = 113.11(4) Å for a calculated density of 1.13 g/cm^3. After a number of solution attempts, tangent formula approaches and Fourier techniques produced an initial model for the molecule. The model is currently being refined, using full matrix least-squares techniques; the present R factor is 11.2%. From the above experiments it was determined that mevinolinic acid has the structure and absolute configuration shown in Fig. 1. Complete structural details for mevinolin and its amide derivative will be reported when refinements are completed.

Inhibition of HMG-CoA Reductase. Assay conditions, including order of addition, were established for investigating the kinetics of inhibition of HMG-CoA reductase such that the assay was linear in the presence or absence of inhibitor and at all levels of HMG-CoA for at least 5 min. This was of particular importance because it was found in preliminary experiments (data not shown) with sodium salts of the hydroxy acids corresponding to either mevinolin or ML-236B that there was a rapid decrease in rate if the reaction was initiated with enzyme. In the absence of inhibitor the order of addition of enzyme did not influence the rate of reaction. It was found, however, that when the enzyme was incubated for 5 min with HMG-CoA and the inhibitor, and the reaction was then initiated with NADPH, linear kinetics were obtained. Presumably this allowed for an equilibrium to be established between inhibitor, substrate, and enzyme. Under these conditions, both inhibitors behaved in a competitive manner with respect to HMG-CoA (Fig. 2), as determined by the method of Dixon (25). In a separate experiment the K_m for HMG-CoA was found to be 4.0 μM. The K_i for sodium mevinolinate was computed to be 0.64 nM, and the K_i for the sodium salt of the hydroxy acid form of ML-236B was 1.4 nM. Thus the metabolite isolated from *A. terreus*, mevinolinic acid, is more than two times as active as the salt of ML-236B as an inhibitor of HMG-CoA reductase.

Acute Inhibition of Cholesterol Synthesis in Rats. It has not been possible to demonstrate hypocholesterolemic activity in rats with ML-236B except after treatment with Triton WR-1334 (26). However, after a single oral dose of ML-236B, cholesterol synthesis from [^{14}C]acetate in normal rats was markedly inhibited 2–8 hr subsequent to treatment (14). Preliminary experiments with both sodium mevinolinate and ML-236B as the sodium salt of its hydroxy acid form established that the onset of inhibitory activity was rapid in rats. The data are summarized in Fig. 3. One hour after administration of the

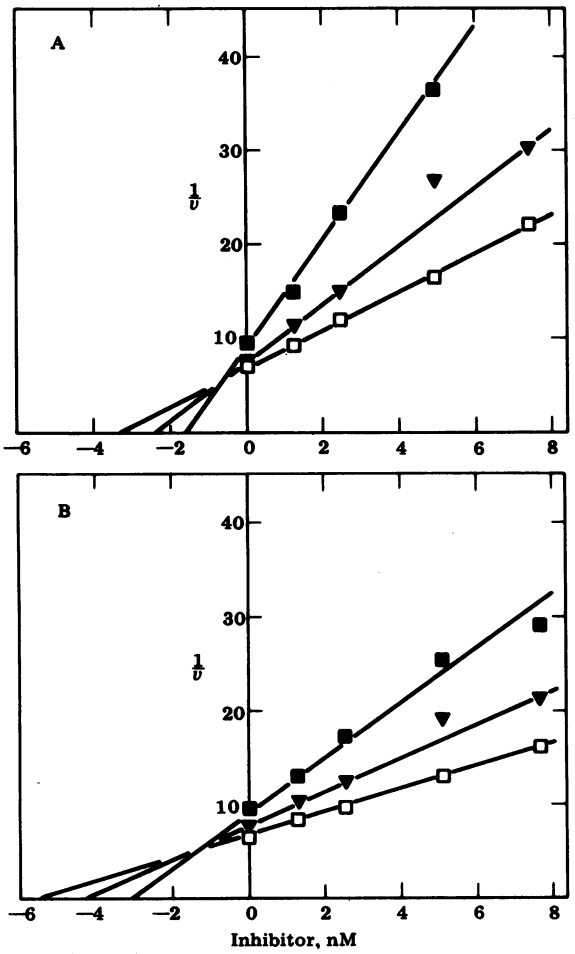

FIG. 2. Dixon plots of inhibition of HMG-CoA reductase by the sodium salts of mevinolinate (*A*) and ML-236B (*B*). Reactions took place in the presence of three different DL-HMG-CoA concentrations: ■, 17.5 μM; ▼, 34.8 μM; □, 52.1 μM.

compounds, [^{14}C]acetate (80 μCi/kg) was injected intraperitoneally, and 50 min later plasma [^{14}C]cholesterol was determined as a measure of sterol synthesis. Sodium mevinolinate

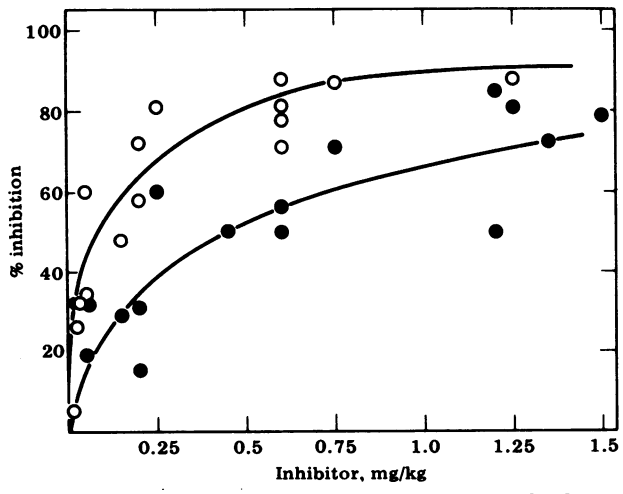

FIG. 3. Acute inhibition of cholesterol synthesis from [^{14}C]acetate in rats after a single oral dose of either sodium mevinolinate (○) or the sodium salt of ML-236B (●). Shown are the results of a number of experiments carried out at different times. Each point is the average of the percent inhibition in 10 rats compared to controls assayed on the same day.

was approximately 6 times as active as the sodium salt of ML-236B, with 50% inhibitory doses of 0.046 and 0.29 mg/kg, respectively.

Hypocholesterolemic Activity in Dogs. In order to ascertain the chronic effect of mevinolin on plasma cholesterol levels in nonrodents, studies were carried out in dogs. As seen in Table 1, dogs treated with mevinolin in their diet at 8 mg/kg per day for 5 days showed a significant decrease in plasma cholesterol (14.1%). After 21 days a 29.3% decrease was seen. In a similar study, Tsujita *et al.* (16) demonstrated that ML-236B at 20 mg/kg per day for 12 days resulted in a 30.4% decrease in plasma cholesterol; this can be compared to a 24.2% decrease with mevinolin at 8 mg/kg per day for 12 days (Table 1). This indicates that comparable decreases in plasma cholesterol in dogs may be obtained with mevinolin at doses significantly lower than those required with ML-236B.

An examination of the lipoprotein profile of dogs treated with mevinolin indicated that low density lipoprotein (LDL) was preferentially lost (data not shown). Because in dogs the predominant lipoprotein is high density lipoprotein (HDL), under conditions in which there was extensive lowering of plasma cholesterol, the absolute amount of HDL was also somewhat decreased. The net result was a decrease in LDL/HDL ratio of approximately 50%.

DISCUSSION

The present report summarizes studies on the isolation, structural characterization, and biochemical properties of an inhibitor of HMG-CoA reductase, mevinolin, which was produced by cultures of a strain of *A. terreus*.

Mevinolin (Fig. 1, structure 1) is a member in the family of substituted hexahydronaphthalene lactones, which interfere with the biosynthesis of mevalonic acid by inhibition of HMG-CoA reductase. This family of natural products includes nuclear variants of compactin (ML-236B) that lack the 2-methyl-1-oxobutyl and 2-methyl-1-oxobutoxy groups (ML-236A and C, respectively). The active form of all of these compounds is the hydroxy acid (opened lactone) structure. In anticipation of additional structural variants of both natural and possible semisynthetic origin, we propose the name mevinic acid (structure 3) for the parent hydroxy acid (1,2,6,7,8,8a-hexahydro-β,δ-dihydroxy-1-naphthaleneheptanoic acid). Thus, mevinolin is 2β,6α-dimethyl-8α-(2-methyl-1-oxobutoxy)-mevinic acid lactone and ML-236B (compactin) is 2β-methyl-8α-(2-methyl-1-oxobutoxy)mevinic acid lactone or 6-demethylmevinolin.

The structure and absolute configuration of the lactone form of mevinolinic acid, mevinolin, were determined by UV and infrared absorption, mass spectral analysis, ^{1}H and ^{13}C NMR analysis, and x-ray analysis. Recently Endo (27) reported on the isolation of monacolin K from a *Monascus* species. From the physical data presented, it appears that mevinolin may be identical or very similar to monacolin K.

Table 1. Mevinolin-induced decrease in plasma cholesterol levels in dogs

Day	% decrease in plasma cholesterol
5	14.1 ± 5.3*†
12	24.2 ± 3.8‡
21	29.3 ± 2.5*§

Decreases are given ± SEM.
* Significantly different from each other ($P < 0.02$).
†‡§ Significantly different from four untreated controls at $P < 0.05$, $P < 0.002$, and $P < 0.001$, respectively.

Mevinolinic acid is a highly potent inhibitor of HMG-CoA reductase. The additional methyl group at the 6α-position confers a 2- to 3-fold enhancement of the intrinsic inhibitory activity of mevinolin compared to ML-236B. This was indicated by kinetic analysis with HMG-CoA reductase. An even greater enhancement of activity was demonstrated in the acutely treated rat assay of cholesterol biosynthesis. This may be due to slower metabolism of mevinolin.

Variable results have been obtained with ML-236B with respect to its hypocholesterolemic activity. It appears to be effective in lowering plasma cholesterol at relatively high doses in dogs (16) and monkeys (17) and at much lower doses in humans (18). However, in rats receiving multiple doses of the compound no effect on plasma cholesterol was noted (7, 10). This lack of effectiveness of ML-236B in the rat could be attributed at least in part to greatly increased levels of HMG-CoA reductase (11, 15), which compensated for the inhibitory activity of ML-236B. In tissue culture, incubation with ML-236B also results in greatly increased levels of reductase (7, 11). Similar results have been obtained with mevinolin in tissue culture and in rats (unpublished observations).

Nevertheless, the apparent potency and efficacy of ML-236B in humans (18) and the comparatively greater activity of mevinolin reported here in *in vitro* and animal models, indicate the potential of mevinolin as a therapeutic agent for the treatment of hypercholesterolemia.

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