Biotransformation of Cerivastatin in Mice, Rats, and Dogs in Vivo

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ABSTRACT:

Biotransformation of cerivastatin was investigated in mice, rats, and dogs in vivo using the ¹⁴C-labeled drug. Marked species differences exist, both in pathways and extent of cerivastatin metabolism. Unchanged drug, together with its lactone, predominates in dog plasma and represents 40% of the dose in the excreta, whereas in rat bile they account for approximately 10% of the dose. In mice, the drug is metabolized rapidly and almost completely. Biotransformation of cerivastatin occurs by three distinct phase I routes and by phase II conjugation with sugar-type moieties and taurine. Phase I routes are demethylation of the pyridinyl methyl ether, β-oxidation of the 3,5-dihydroxy acid side chain, and reductive removal of the side chain 3-hydroxy group. In dogs, demethylation is the dominating phase I biotransformation. Phase II conjugation is equally important. In dog bile, different regioisomeric drug glucuronides and the benzylic glucuronide and glucoside conjugate of the demethylated drug were found. In rats, besides demethylation, β-oxidation of the dihydroxy acid side chain—followed by reductive removal of the 5-hydroxy group—is the major reaction. The resulting pentenoic acid derivatives are observed in plasma and liver homogenate. These metabolites are subsequently conjugated with taurine and excreted in the bile. This metabolic sequence is also important in mice. Furthermore, only in mice, cerivastatin is subject to reductive removal of the 3-hydroxy group, together with demethylation. The 5-hydroxyheptenoic acids formed predominate in plasma and liver homogenate, whereas the corresponding taurine conjugates are excreted in the bile.

Cerivastatin, sodium (E)-(+)-(3R,5S)-7-[4-(4-fluorophenyl)-2,6-diisopropyl-5-(methoxymethyl)-pyrid-3-yl]-3,5-dihydroxyhept-6-enoate (BAY w 6228), is used for the treatment of hypercholesterolemia (Angerbauer et al., 1994). It was shown to be a highly potent inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis in vitro (Corsini et al., 1996). It was also demonstrated that cerivastatin effectively inhibits cholesterol synthesis in vivo (Bischoff and Petzina, 1992).

Studies on the in vitro metabolism of cerivastatin by human liver microsomes (Boberg et al., 1997) suggested that O-demethylation and stereoselective hydroxylation in the 6'-isopropyl group are the main biotransformation reactions in man (for numbering assignment, see scheme 1).

This paper describes the metabolism of cerivastatin in mice, rats, and dogs in vivo. Single metabolites were isolated from rat, dog, and mouse bile, as well as from mouse hepatocyte incubations, and their structures were elucidated.

Materials and Methods

Radiolabeled Compound and Reagents. Sodium (E)-(+)-(3R,5S)-7-[4-(4-fluorophenyl)-2,6-diisopropyl-5-(methoxymethyl)-pyrid-3-yl]-3,5-dihydroxyhept-6-enoate ([¹⁴C]cerivastatin; M. Radtke, R. Angerbauer, manuscript in preparation) with a specific activity of 2.28 –2.43 MBq/mg was used. The radiochemical purity was at least 97% when determined by HPLC.

All reagents and chemicals used for analytical procedures were of analytical or HPLC grade and purchased from E. Merck, Darmstadt, Germany. All reagents and chemicals used for chemical synthesis were of synthetic grade and purchased from Aldrich-Chemie, Steinheim, Germany. Beta-glucuronidase/sulfatase from Helix pomatia and β-glucosidase from almonds were purchased from Sigma, Taufkirchen, Germany.

Chemical Syntheses. The synthesis of desmethyl cerivastatin (metabolite M-1 reference) and of the enantiomerically pure metabolite M-23 and M-24...
reference compounds, hydroxylated in the 6'-isopropyl group, has been described previously (Boberg et al., 1997; Angerbauer et al., 1993). M-1 was transformed into the corresponding lactone M-22 reference using hydrochloric acid. The 5-keto derivative of cerivastatin (metabolite M-7 reference) was synthesized from cerivastatin methyl ester by manganese dioxide oxidation and subsequent ester hydrolysis. The pentadienoic acid 2 (metabolite M-29 reference) and the corresponding taurine conjugate 3 (metabolite M-19 reference) were obtained from an intermediate of the cerivastatin synthesis (Angerbauer et al., 1994) as outlined in scheme 2. Compound 3 readily cyclized to a tricyclic dihydrobenzoxideqinoline, which was separated in small amounts during purification of 3. It was similarly isolated as metabolite M-20 during purification of metabolite M-19 (scheme 3).

Trans-(E)-6-[2,6-diisopropyl-4-(4-fluorophenyl)-5-hydroxymethyl-pyrid-3-yl]-ethyl-3,4,5,6-tetrahydro-4-hydroxy-2H-pyran-2-one (metabolite M-22 reference). A solution of 1.494 g (3.2 mmol) of sodium ethrythro-(E)-7-[2,6-diisopropyl-4-(4-fluorophenyl)-5-hydroxymethyl-pyrid-3-yl]-3,5-dihydroxyhept-6-enoate (desmethyl-cerivastatin) in 150 ml water was adjusted to pH 4 using 1 N HCl and extracted with EtOAc. The EtOAc phase was dried with Na2SO4 and concentrated under vacuum. The residue was dissolved in 100 ml absolute toluene, and 40 g molecular sieves 4 Å was added and heated under reflux overnight. It was subsequently filtered, concentrated under vacuum, and the residue crystallized using PE, yielding 0.808 g (59%) product.

Methyl (E)-5-[2,6-diisopropyl-4-(4-fluorophenyl)-5-methoxymethyl-pyrid-3-yl]-prop-2-enal (metabolite M-23 reference). To a solution of 2.3 g (5.2 mmol) of methyl-penta-2,4-dienoate in 150 ml water was adjusted to pH 5.9 using 0.25 N NaOH solution and the aqueous residue was freeze-dried to yield 45 mg (94%) product.

Methyl (E)-5-[2,6-diisopropyl-4-(4-fluorophenyl)-5-methoxymethyl-pyrid-3-yl]-penta-2,4-dienoate. To a solution of 355 mg (1 mmol) (E)-3-[4-(4-fluorophenyl)-2,6-diisopropyl-5-methoxymethyl-pyrid-3-yl]-prop-2-enol 1 in 10 ml toluene, 334 mg (1 mmol) methoxybenzyl methylene triphenylphosphorane was added, and the mixture was stirred for 2 days at room temperature. After concentrating in vacuo, the mixture was chromatographed on silica, using EtOAc/PE 5:95 to give 405 mg (99%) product.

1H NMR (CDCl3): δ = 1.26 (d, 6H); 1.31 (d, 6H); 1.31 (m, 2H); 3.71 (s, 3H); 4.06 (s, 3H); 5.71 (d, 1H); 5.94 (dd, 1H); 6.61 (d, 1H); 7.0 –7.2 (5H) ppm.

1H NMR (CDCl3): δ = 1.22 (d, 6H); 1.32 (d, 6H); 3.18 (s, 3H); 3.31 (m, 2H); 4.29 (m, 1H); 5.23 (dd, 1H); 6.31 (d, 1H); 7.0 –7.3 (m, 5H) ppm.

To a solution of 1.27 g (3.2 mmol) of the pentadienoic acid 2 and 0.59 g (3.3 mmol) HONB in 10 ml dry THF, a solution of 0.68 g (3.3 mmol) DCC in dry dioxane was added at 0°C, and the mixture was stirred for 20 min at the same temperature, then for 40 min at room temperature. The resulting dicyclohexylurea was filtered off, and the filtrate was concentrated in vacuo. The residue was dissolved in 10 ml dry dioxane, and a solution of 0.4 g (32 mmol) taurine in 32 ml 0.1 N NaOH solution was added at room temperature. After being stirred for 8 hr, the mixture was evaporated in vacuo, and the residual aqueous solution was applied to a column packed with 11 g Amberlite® IR120 (H⁺-form) resin (Aldrich). The water eluate was freeze-dried, yielding 516 mg (32%) of crude 3. Further purification of 9.8 mg crude product by HPLC on a Nucleosil® C18 column (Machery + Nagel, Dueren, Germany), using 0.05 N ammonium acetate/acetonitrile and water/acetonitrile gradient elution, yielded 0.6 mg pure 3.

Measurement of Radioactivity. Radioactivity of liquid samples was measured at 13°C in a Canberra Packard TriCarb® 2500 TR liquid scintillation spectrometer (Groningen, The Netherlands) with automatic quench correction by the external standard channel ratio method, using Ultima Gold® (Canberra Packard) as scintillation cocktail.

Spectroscopic Methods. FAB mass spectra were recorded on a Finnigan MAT 8430 mass spectrometer (Bremen, Germany), using glycerol as matrix and xenon as bombardment gas. GC/MS analyses were performed on the same instrument, using a Varian 3700 gas chromatograph (Darmstadt, Germany) equipped with a 30 m Durabond® DB1 fused silica capillary (film thickness 0.25 μm; Hewlett-Packard, Waldbronn, Germany) and operated in a temperature-programming mode. Metabolites were analyzed as their trimethylsilyl derivatives, which were formed by treatment with MSTFA at 80°C for 30 min.

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**Scheme 2. Synthesis of metabolites M-29 and M-19 reference.**

Reagents: (a) Ph,PCHCOOCH3; (b) NaOH; (c) HONB/DCC, H2NCH2CH2SO3H.
The splitless injection technique, according to Grob and Grob (1974), was used with direct coupling and EI ionization at 70 eV and/or CI condition with ammonia as reactant gas. Positive mode ESI mass spectra were recorded by LC/MS using an ABI 140B HPLC system (Applied Biosystems, Inc., Foster City, CA) coupled with a PE/Sciex/API III mass spectrometer (Perkin Elmer Sciex Instruments, Thornhill, Ontario, Canada). A 5-μm Supelcosil™ LC-18 column (250 × 2.1 mm) was eluted with a step gradient from 50% 10 mM ammonium acetate to 90% acetonitrile over a period of 30 min. The column effluent (250 μl/min) was connected to the Ionspray® interface, with a splitting ratio of 1:10. 1H NMR spectra of isolated metabolites and reference compounds were recorded at 500 MHz on a Bruker AMX 500 NMR spectrometer (Bruker, Rheinstetten, Germany) using methanol-d4 (99.96% deuterium content) as solvent.

Chromatographic Methods. HPLC. Analytical HPLC of plasma, bile, urine, and liver extracts was performed on a HP 1090 M liquid chromatograph with diode array detection (Hewlett-Packard) and online radioactivity monitor Ramona® (Raytest, Straubenhardt, Germany) using methanol-d4 (99.96% deuterium content) as solvent.

For metabolite profiling, a 5-μm LiChrospher® RP8 column (250 × 4 mm) was eluted with a step gradient from 70% solvent A (0.1% aqueous TBAH, adjusted to pH 5 with 0.1% aqueous sodium hydroxide or PIC A® reagent) to 100% solvent B (20% solvent A, 80% acetonitrile) within 130 min. The flow rate was 1.3 ml/min and the oven temperature was 40°C. Alternatively, step gradient elution from 100% 0.2% phosphoric acid to 100% acetonitrile within 63 min was performed. The HPLC method used for LC/MS was applied to metabolite profiling of mouse bile.

Bile samples were analyzed directly. Urine samples were concentrated prior to analysis by applying a 3- to 10-ml aliquot to a Bond Elut® C8 cartridge (ICT, Frankfurt, Germany), which was preconditioned with acetonitrile and water. After being washed with 0.1% aqueous phosphoric acid or water, the cartridge was eluted with acetonitrile (recoveries: 0.5 hr, 100%; 1 hr, 93%; 3 hr, 81%; 7 hr, 76%; 24 hr, S C H E M E 3. Metabolic pathways of cerivastatin in dogs.

The splitless injection technique, according to Grob and Grob (1974), was used with direct coupling and EI ionization at 70 eV and/or CI condition with ammonia as reactant gas. Positive mode ESI mass spectra were recorded by LC/MS using an ABI 140B HPLC system (Applied Biosystems, Inc., Foster City, CA) coupled with a PE/Sciex/API III mass spectrometer (Perkin Elmer Sciex Instruments, Thornhill, Ontario, Canada). A 5-μm Supelcosil™ LC-18 column (250 × 2.1 mm) was eluted with a step gradient from 50% 10 mM ammonium acetate to 90% acetonitrile over a period of 30 min. The column effluent (250 μl/min) was connected to the Ionspray® interface, with a splitting ratio of 1:10. 1H NMR spectra of isolated metabolites and reference compounds were recorded at 500 MHz on a Bruker AMX 500 NMR spectrometer (Bruker, Rheinstetten, Germany) using methanol-d4 (99.96% deuterium content) as solvent.

Chromatographic Methods. HPLC. Analytical HPLC of plasma, bile, urine, and liver extracts was performed on a HP 1090 M liquid chromatograph with diode array detection (Hewlett-Packard) and online radioactivity monitor Ramona® (Raytest, Straubenhardt, Germany), connected via an analog/digital converter that transformed dpm values into mV units.

For metabolite profiling, a 5-μm LiChrospher® RP8 column (250 × 4 mm) was eluted with a step gradient from 70% solvent A (0.1% aqueous TBAH, adjusted to pH 5 with 0.1% aqueous sodium hydroxide or PIC A® reagent) to 100% solvent B (20% solvent A, 80% acetonitrile) within 130 min. The flow rate was 1.3 ml/min and the oven temperature was 40°C. Alternatively, step gradient elution from 100% 0.2% phosphoric acid to 100% acetonitrile within 63 min was performed. The HPLC method used for LC/MS was applied to metabolite profiling of mouse bile.

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GERMANY). Hepatocytes were incubated under an atmosphere of 95% O2/5% CO2 at a cell density of 10^8 cells/80 ml for 4.5 hr. Enzymatic reactions were stopped with a cocktail of the retroorbital venous plexus or exsanguination after incision of the carotid artery. Bile was obtained from bile duct–cannulated mice using a Pasteur pipette. All sample amounts or radioactivity concentrations. Blood was collected by puncture of the retroorbital venous plexus or exsanguination after incision of the carotid artery. Bile was obtained from bile duct–cannulated mice using a catheter tied into the proximal part of the common bile duct. The distal end and the cystic bile duct were ligated. Surgery was performed in anesthetized mice (ketamine, xylazine), and [14C]cerivastatin was administered to conscious mice by intracarotid injection.

**Animal Experiments.** The studies performed on male and female Wistar rats as well as female beagle dogs have been described elsewhere (Steinke et al., 1996). The studies in mice were performed in male B6C3F1 mice (Bomholtgard, Ry, Denmark) weighing approximately 30 g. [14C]Cerivastatin was dissolved in PBS, pH 7.4, containing 1.5% polysorbat 80 (Tween 80; Fuji) was evaluated in the TINA® software (Raytest, Straubenhardt, Germany) (Klein and Clark, 1993).

**Preparation and Incubations of Mouse Hepatocytes.** Hepatocytes were isolated from the livers of male B6C3F1 mice (30 g) by the two-step collagenase perfusion method of Berry and Friend (1969) with modifications described by Moldeus et al. (1978). Mice were anesthetized with pentobarbital (60 mg/kg), and after an intravenous dose of heparin (2000 U · kg^-1), the liver was perfused at 37°C with oxygenated Ca²⁺-free Hank's buffer with collagenase type A (0.12%). Hepatocytes were freed from the liver and purified by gravity sedimentation, using the incubation buffer as medium. Finally, the hepatocytes were resuspended in the incubation buffer. Cell yield was assessed by hemocytometer (0.1 ml). Cell viability was determined using the trypan blue exclusion method. The number of cells recovered was quantitated using a Neubauer hemocytometer (0.1 ml; Brand, Weinheim, Germany). Hepatocytes were incubated under an atmosphere of 95% O2/5% CO2 in 250 ml roundbottom flasks rotated continuously in a water bath with a shaking speed of 120 cycles/min at 37°C. To produce metabolites for structure elucidation, an incubation with [1^14C]cerivastatin (10 μg/ml) was conducted at a cell density of 10^6 cells/ml for 4.5 hr. Enzymatic reactions were stopped by addition of an equal volume of ice-cold acetone.

**Isolation and Purification of Metabolites.** Pooled 0- to 7-hr rat bile fractions from 7 animals were adjusted to pH 4 with 8.5% aqueous phosphoric acid and centrifuged. The supernatant was applied to Mega Bond Elut M-1 cartridges (ICT, Frankfurt, Germany) and metabolite mixtures were obtained by gradient elution with water/acetonitrile. From these primary fractions, single metabolites were isolated by HPLC using acetonitrile/water gradient elution. Fractions containing metabolites M-1 and M-15 to M-21 were isolated. The combined 0- to 7-hr bile fractions from one dog were worked up similarly, yielding metabolites M-1 to M-11.

Mouse hepatocyte incubation mixtures were centrifuged and the supernatant was discarded. After adjustment to pH 4.6 with 8.5% phosphoric acid, Bond Elut® workup and subsequent HPLC purification were performed as described above. Metabolites M-19, M-27, M-29, M-30, and M-32 were isolated. The 0- to 7-hr bile fractions from 9 mice were pooled, diluted with an equal volume of water, and adjusted to pH 4.75, using acetate buffer pH 4.66/10% aqueous acetic acid. After addition of β-glucuronidase/arylsulfatase from H. pomatia (Serva, Heidelberg, Germany), the mixture was incubated at 37°C and 75 rpm for 24 hr. Workup as described above yielded purified samples of metabolites M-19 and M-25 to M-28. The structures of isolated metabolites were elucidated by 1H-NMR spectroscopy and mass spectrometry.

**Enzymatic Cleavage of Glucuronides and Glucosides.** Glucuronide samples were incubated at 37°C overnight in 0.2 ml buffer, pH 4.52, containing β-glucuronidase/arylsulfatase (1000 Fishman and 10000 Roy units). Glucoside samples were treated similarly at pH 5.0 with 5 mg β-glucosidase (0.2 units) from almonds. The reaction mixtures were analyzed by HPLC.

## Results

**Excretion of Radioactivity.** After administration of [1^14C]cerivastatin via different routes, total radioactivity was excreted almost exclusively with the bile/feeces in rats and dogs (Steinke et al., 1996) and also in mice. After an oral or intraduodenal dose of 2 mg/kg, less than 0.5% was found in the urine of male rats (Steinke et al., 1996), whereas 1.5% was found after an oral dose of 20 mg/kg. Thus the latter was used for metabolite profiling. For similar reasons, dog urine was only analyzed after oral drug administration. Excretion data are summarized in table 1.

**Metabolite Profiles. Plasma and Liver.** Similar metabolite profiles were observed upon HPLC analysis of mouse plasma and liver extracts 0.5 hr as well as 4 hr after oral drug administration (table 2). Two major metabolites M-27 and M-28 represent the same metabolic pathway, which is characterized by a formal reduction of the side chain β-position (scheme 4). The corresponding taunine conjugates M-25 and M-26 were less pronounced. A second important pathway is characterized by β-oxidation, elimination, and reduction processes at the dihydroxyheptenoyl side chain. In addition to the aglycones M-21 and M-30, the taunine conjugates M-15, M-16, M-18, and M-19 were found, thereby indicating the importance of this conjugation reaction in cerivastatin metabolism in mice. Unchanged cerivastatin and the primary deethylated metabolite M-1 were not detected by HPLC even 0.5 hr after dosing.

**TLC analysis with radioluminographic detection provides a higher sensitivity as compared with HPLC with offline radioactivity counting. Thus, upon TLC analysis, 2%–3% cerivastatin and 2% M-1 were detected in mouse plasma. In liver extracts, similar amounts were found (3%–4% and 2%–3%, respectively; data not shown). TLC**
analysis was hampered by the fact that all taurine conjugates remained at the start of the plate and therefore could not be quantified individually. The aglycone of metabolite M-19, the pentadienoic acid M-29, co-migrated with a TLC spot, which represented 13% of radioactivity in the 4-hr plasma extract, but was not identified upon HPLC analysis. Therefore its identification remains tentative.

The metabolite profiles in rat plasma and liver extracts were established up to 24 hr after oral drug administration by TLC. Both showed similar time-dependent changes (table 3). Up to 3 hr after dosing, unchanged cerivastatin predominated; later, metabolite M-21 was the main metabolite identified. Metabolites M-1 and M-30 were minor, and a considerable amount of total radioactivity remained at the start of the TLC plates. HPLC analysis of single 4-hr liver samples from a different study showed that the taurine conjugates M-15, M-16, M-18, and M-19 together accounted for 12% of total radioactivity, each being almost equally important. Cerivastatin and metabolites M-1 and M-21 were found in similar amounts as by TLC (data not shown).

In dog plasma, unchanged cerivastatin is the major component detected up to 2 hr after oral drug administration (table 4). Besides metabolite M-1, the drug lactone M-8 was found, probably because of acidic conditions during sample workup.

**Bile.** At least 21 metabolites were detected in mouse bile (fig. 1). Unchanged cerivastatin was only found in traces (table 5). The two different biotransformation pathways involving the side chain were found to be equally important. Metabolites M-25, M-26, M-27, and M-28 lacking the 3-hydroxy group, together accounted for 28.2% of dose. On the other hand, M-15, M-16, M-18, M-19, and M-20, which exhibit a side chain shortened by two carbon atoms, represented 32.9%.

In the 0- to 24-hr bile fractions of male and female bile duct-cannulated rats, 7 major and several minor metabolites were detected (fig. 2). Unchanged cerivastatin was balanced with 7.2% and 8.2% of dose for males and females, respectively. The drug lactone M-8 accounted for less than 2% of dose in both sexes. A sex difference was observed with respect to primary demethylation. Metabolite M-1 was the main component in bile from female animals but was rather minor in male rat bile (18.7% and 5.5% of dose, respectively). On the other hand, biotransformation of the dihydroxyheptenoic acid side chain appears to be more important in male rats. The amounts of metabolites M-16, M-18, and M-19 were 2–3 times higher in bile from males as

![Scheme 4](image_url)

**Scheme 4.** Metabolic pathways of cerivastatin in mice and rats.

<table>
<thead>
<tr>
<th>Table 2: Metabolite profiles in mouse plasma and liver extracts after oral administration of 2 mg/kg cerivastatin</th>
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<td><strong>Metabolite (%)</strong></td>
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*Established by HPLC; experimental conditions were as described in “Materials and Methods.”

*Values are given in % of extract.

*Not detected.
compared to females. No major time-dependent changes in the metabolite profiles in rat bile were observed. In contrast, the number of metabolites excreted with dog bile increased with time (fig. 3). Whereas in the early sampling intervals, unchanged drug, the drug lactone M-8 and metabolite M-1 predominated, 24 minor metabolites were particularly excreted with the 7- to 24-hr bile sample. Up to 48 hr after dosing, cerivastatin and drug lactone M-8 together accounted for 40.6% of dose. Besides 13.1% of M-1 found, only the 5-glucuronide M-3 and the M-1 glucoside M-5 were found in considerable amounts (3.9% and 5.2%, respectively). All other known and unknown metabolites were balanced with approximately 1% of dose or less (table 5). Besides demethylation and conjugation with glucuronic acid (M-2, M-3, M-4, M-6) and glucose (M-5), hydroxylation at the 2-position of the side chain was a minor biotransformation reaction in dogs as indicated by metabolites M-9, M-10, and M-11. In the ion-paired HPLC gradient used, all 2-hydroxy metabolites exhibited a broad peak shape. Therefore unequivocal identification of M-10 and M-11 in native bile was not possible, and M-9 represented a tailing of the M-1 peak (fig. 3, panel B).

Urine. After oral administration of 2 mg/kg [14C]cerivastatin, mice and dogs excreted 3.6%–3.7% of total radioactivity with the urine, and concentrated samples were analyzed by HPLC. However, because in the corresponding male rat study only 0.4% of dose was excreted renally (Steinke et al., 1996), HPLC analysis was not possible. Therefore urine samples from a higher dose study were used for metabolite profiling (table 1).

Unchanged cerivastatin was detected only in traces in mouse and rat urine samples and accounted for less than 10% of radioactivity in dog urine (table 6). The drug lactone M-8 was not present in mouse urine and represented only 1%–2% of radioactivity in rat and dog urine samples.

In mice, the taurine conjugate M-15 was found with almost 20% of total renally excreted radioactivity (table 6). Reductive biotransformation of the side chain was mainly represented by 10% M-25 and 3% M-26 (table 6).

In rat urine, the primary metabolite M-1 represented roughly half of the radioactivity, and the corresponding lactone M-22 contributed
another 4% (table 6). Metabolites M-15 and M-16 together accounted for less than 10%.

In dog urine, metabolite M-1 and its glucuronide M-4 and glucoside M-5 together made up for 60% of radioactivity (table 6), the drug glucuronides M-2, M-3, and M-6 being minor.

**Isolation and Structure Elucidation of Metabolites.** Structure elucidation of metabolites M-1 and M-22 has been described previously (Boberg et al., 1997). Metabolites M-2, M-3, M-4, M-5, M-6, M-9, M-10, and M-11 were isolated from dog bile. M-15, M-16, M-18, M-19, and M-21 were isolated from rat bile, together with M-25, M-26, M-27, and M-28 from mouse bile. For isolation of metabolites M-29 and M-30, [14C]cerivastatin was incubated with freshly isolated mouse hepatocytes. Metabolite M-32, which was not found in any of the ex vivo samples analyzed, was also isolated from these mouse hepatocyte incubations. Identification of metabolites in plasma, urine, and bile samples was performed by HPLC co-elution with purified metabolite samples. The structures of metabolites were elucidated by combined GC/MS, FAB/AMS, combined LC/ESI/MS, and 1H-NMR spectroscopy. The NMR and mass spectra of the individual metabolites are not discussed in detail, essential data are summarized in table 7.

**Metabolite M2.** The EI mass spectrum of the trimethylsilylated metabolite yielded a molecular ion at m/z 1067, which is in accordance with the hexakis-TMS derivative of a drug glucuronide. The elimination of tetrakis-TMS glucuronic acid (m/z 585) and the ions m/z 375 and 391 are characteristic for persilylated glucuronides (fig. 4).

Additional evidence for the drug glucuronide structure of M-2 was obtained from enzymatic cleavage. Treatment with β-glucuronidase from H. pomatia resulted in complete conversion to the unchanged drug. Incubation of M-2 with buffer pH 8.9 at room temperature resulted in enhanced decomposition to several compounds, a known characteristic of acyl glucuronides (Faed, 1984).

**Metabolite M-3.** The CI mass spectrum of the trimethylsilylated compound yielded an intense MH+ ion at m/z 1068, in agreement with the hexakis-TMS derivative of a drug glucuronide. The corresponding EI mass spectrum showed in addition a very weak fragment M-CH3 at m/z 1052, the bis-TMS-aglycone ion m/z 602, and typical fragments of persilylated glucuronides at m/z 375, m/z 391, and m/z 217. The ion m/z 233 is characteristic for TMS derivatives of β-hydroxy fatty acids (Petersson, 1970). The 1H NMR spectrum of M-3 showed high similarity to that of the unchanged drug. Additionally, the anomic proton appeared as a doublet at δ = 4.02 ppm, being a typical chemical shift for alkyl β-glucuronides. The downfield shift of the methine proton H-5 (+0.26 ppm), in comparison with the corresponding signal of the drug (Boberg et al., 1997), proved that the 5-hydroxyl group was glucuronidated. In accordance with the proposed structure, treatment of M-3 with β-glucuronidase from H. pomatia resulted in the exclusive formation of cerivastatin. On the contrary, metabolite M-3 was stable toward treatment with 0.1 N HCl. Under these conditions, cerivastatin is converted in part to its lactone. Thus, glucuronidation of the 5-hydroxyl function does not result in an enhanced electrophilic reactivity at this carbon center. M-3 was also stable toward buffer pH 9. Separation of metabolites M-4 and M-5 was difficult in every HPLC system used, indicating structural similarity (fig. 3).

**Metabolite M-4.** The EI mass spectrum of the trimethylsilylated metabolite yielding a molecular ion at m/z 1125 and two further characteristic ions at m/z 553 and m/z 464 indicated a heptakis-TMS derivative of desmethyl cerivastatin (M-1). In the 1H NMR spectrum, the AB system of the CH2OH-group shifted from 4.08 and 4.81 ppm, indicating the position for glucuronidation. A new anisotropic effect is characteristic of glucuronidation of the CH2OH group. The position of the glucuronoi moiety was further confirmed by the chemical shift of the protons H-2α, H-2β, H-3, H-4α, and H-5, which were nearly identical to those in the spectrum of metabolite M-1. The anomic proton at δ = 3.98 ppm with J = 7.8 Hz indicated an alkyl glucuronide as β-anomer.

**Metabolite M-5.** The EI mass spectrum after trimethylsilylation exhibited the same aglycone ions m/z 553 and m/z 464 as M-4, a molecular ion at m/z 1111, and additional ions m/z 361 and m/z 377, typical for trimethylsilylated hexosides, instead of the sugar ions m/z 375 and m/z 391 found for glucuronides. The strong ion at m/z 331 is interpreted as a rearrangement of the ion at m/z 361 under loss of formaldehyde. The 1H NMR spectrum was very similar to that of metabolite M-4. The AB system of the CH2O group appeared at δ = 4.08 and 4.81 ppm, indicating the position for glucosidation. A new ABX system (the X part is superimposed) at δ = 3.63 and 3.79 ppm with JAB = 12.0 Hz is typical for the methylene group of a glucoside. The anomic proton at δ = 4.01 ppm with J = 7.8 Hz indicated the β-glucoside form.

Treatment of metabolite M-4 with β-glucuronidase/sulfatase from H. pomatia furnished metabolite M-1 as the only reaction product, whereas metabolite M-5 was stable toward β-glucuronidase. On the contrary, M-5 was transformed into M-1 using β-glucosidase from almonds.

**Metabolite M-6.** The EI mass spectrum of the trimethylsilylated third drug glucuronide showed a very weak molecular ion (m/z 1067), the persilylated aglycone ions m/z 585 and 602, and—as base peak—the oxonium ion m/z 375. The ion m/z 428 with the plausible structure Aryl-CH=CH-CH=O-+ TMS indicated the non-glucuronidized 5-position in accordance with the proposal of a 3-O-glucuronide. Beta-glucuronidase cleavage of M-6 gave the unchanged drug exclusively. Like metabolite M-3, M-6 was stable toward treatment with 0.1 N HCl; no lactonization or elimination occurred. The different pH stability of the acyl glucuronide M-2, as compared with the alkyl glucuronides M-3 and M-6, further supports the structures proposed.
Metabolite M-9. The EI mass spectrum after trimethylsilylation gave a molecular weight of 821 Da, consistent with the pentakis-TMS derivative of a hydroxylated desmethyl cerivastatin. The fragment ions \( m/z \) 512 and \( m/z \) 486, representing partial structures \( G \) and \( J \), respectively (fig. 5), indicated that the additional hydroxy function was introduced neither in the 4-position of the side chain nor in the aromatic ring or in the isopropyl groups. The only possibility left was hydroxylation in the \( \alpha \)-position of the side chain. The \(^1\)H NMR spectrum showed the intact isopropylc, olefinic, and aromatic moieties. The proton \( H-5 \), appearing with the same chemical shift and multiplicity as in the spectrum of metabolite \( M-1 \), indicated an intact methylene group in 4-position to the carboxylic group. Together with signals of the intact isopropyl groups, these data provide further evidence that the hydroxylation took place in 2-position.

Metabolite M-10. The EI mass spectrum of the silylated metabolite showed the molecular ion at \( m/z \) 1199. The ions \( m/z \) 377 and \( m/z \) 361 indicated a trimethylsilylated glucoside. In analogy to the corresponding ions of derivatized \( M-9 \), the fragment ions \( m/z \) 890 and \( m/z \) 864 were interpreted as structures \( J \) and \( M \), respectively (fig. 5). Treatment of metabolite \( M-10 \) with \( \beta \)-glucosidase from almonds resulted in formation of metabolite \( M-9 \).

Metabolite M-11. Combined GC/MS after trimethylsilylation yielded a CI mass spectrum with a \([M+H]^+\) ion at \( m/z \) 1214. The corresponding EI mass spectrum missing the molecular ion showed fragment ions at \( m/z \) 375 and \( m/z \) 391 characteristic for trimethylsilylated glucuronides. Furthermore, treatment with \( \beta \)-glucuronidase/sulfatase furnished metabolite \( M-9 \) as aglycone.

Metabolite M-15. In the negative mode FAB mass spectrum, the very intense molecular ion at \( m/z \) 491 indicated an even molecular weight. The fragment ions at \( m/z \) 124 and at \( m/z \) 80 were interpreted as the anion of taurine and the radical anion \( SO_3^- \), respectively. The \(^1\)H NMR spectrum confirmed the conjugation with taurine, showing two typical 2H triplets at \( \delta = 2.93 \) and 3.56 ppm (\( J = 6.7 \) Hz). Whereas the aromatic moiety and the two isopropyl groups were unchanged, a pent-4-ene-carboxylic moiety was indicated by two 2H multiplets centered at \( \delta = 2.07 \) ppm and 2.22 ppm and two 1H multiplets at \( \delta = 5.31 \) ppm and 6.09 ppm. The side chain conjugation with taurine was further confirmed by the IR absorption at 1648 and 1547 cm\(^{-1}\), typical for an aliphatic secondary amide.

Metabolite M-16. The negative FAB mass spectrum showed a \([M-2H]^2+\) at \( m/z \) 489. The taurine residue was indicated by the ions \( m/z \) 124 and \( m/z \) 80. In comparison with \( M-15 \), the \(^1\)H NMR spectrum of \( M-16 \) showed two additional signals in the olefinic region instead of the two adjacent methylene groups, a 1H doublet at \( \delta = 5.82 \) ppm, and a multiplet at \( \delta = 6.99 \) ppm. The other signals were nearly identical.

Metabolite M-17. In particular under the influence of light, but also under acidic conditions, metabolite \( M-16 \) underwent a rapid cyclization reaction to yield a tricyclic ring system. This artifact \( M-17 \) was isolated during purification from biological matrix. The negative FAB mass spectrum revealed the same molecular weight as \( M-16 \), indicating an isomerization reaction. The \(^1\)H NMR spectrum of \( M-17 \) showed two...
olefinic and three aromatic protons, strongly indicating the involvement of the aromatic ring in this reaction. Two additional new multiplets at $\delta = 3.68$ ppm (1H) and approximately 2.9 ppm (2H, superimposed by the CH$_2$ triplet of the taurine moiety), interpreted as a new -CHCH$_2$- group, were also in accordance with a tricyclic structure. M-17 was not detected in bile and therefore is seen as a specific decomposition product of M-16 formed during the purification procedure.

Metabolite M-18. The negative FAB mass spectrum indicated the typical taurine fragments m/z 124 and m/z 80 and a molecular weight of 506 Da, which is 14 Da higher than that of M-15. The $^1$H NMR spectrum was nearly identical to that of M-15, with an additional methoxy group ($\delta = 3.15$ ppm) and an upfield shift of the singlet representing the adjacent methylene group.

Metabolite M-19. In the ESI mass spectrum of M-19, the molecular weight of 504 Da was indicated by the [M+Na]$^+$, [M+NH$_4$]$^+$, and [M+H]+ ions, at m/z 527, 522, and 505, respectively. The characteristic fragments at m/z 380, m/z 348, and m/z 311 provided strong evidence for the presence of a pentadienoic acid taurine conjugate. The $^1$H NMR spectrum of M-19 was almost identical to that of M-15, with an additional methoxy group ($\delta = 3.16$ ppm) and the upfield shift of an AB spin system representing the adjacent methylene group.

Metabolite M-20. Cyclization of M-19 was observed during metabolite isolation as well as during purification of the synthetic reference sample. In the ESI mass spectrum of M-20, the [M+Na]$^+$ ion at m/z 527 and the [M+H]+ ion at m/z 505 indicated the same molecular weight as M-19. In contrast to M-19, only one fragment ion at m/z 473, corresponding to the [M+H−CH$_2$OH]+ ion appeared. No fragmentation of the side chain was observed. The $^1$H NMR spectra of M-17 and M-20 showed the same similarities as those of M-16 and

![Fig. 3. Metabolite profile in 0- to 1-hr (A) and 7- to 24-hr (B) bile fraction of a female dog after intraduodenal administration of 2 mg/kg $[^{14}$C]cerivastatin. HPLC was performed as described in “Materials and Methods” with radioactivity detection.](image-url)
### TABLE 7

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Method</th>
<th>Spectroscopic Data</th>
</tr>
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<tbody>
<tr>
<td>M-2, silylated</td>
<td>ES-MS m/z (%): 1067 (1) M⁺; 585 (15) M⁻D⁺</td>
<td>391 (28) A⁺; 375 (19) B⁺; 73 (100) TMS⁺.</td>
</tr>
<tr>
<td>M-3, silylated</td>
<td>ES-MS m/z (%): 1068 (95) [M⁺H]⁺</td>
<td></td>
</tr>
<tr>
<td>M-15, FAB-MS</td>
<td>ES-MS m/z (%): 491 (100) [M⁻H]⁻</td>
<td>124 (26) [NH₄⁺CH₂SO₃⁻]; 80 (55) [SO₃⁻].</td>
</tr>
<tr>
<td>M-16, FAB-MS</td>
<td>ES-MS m/z (%): 489 (84) [M⁻H]⁻</td>
<td>124 (100) [NH₄⁺CH₂SO₃⁻]; 80 (87) [SO₃⁻].</td>
</tr>
<tr>
<td>M-17, FAB-MS</td>
<td>ES-MS m/z (%): 489 (88) [M⁻H]⁻</td>
<td>124 (100) [NH₄⁺CH₂SO₃⁻]; 80 (59) [SO₃⁻].</td>
</tr>
<tr>
<td>M-18, FAB-MS</td>
<td>ES-MS m/z (%): 505 (100) [M⁻H]⁻</td>
<td>124 (27) [NH₄⁺CH₂SO₃⁻]; 80 (51) [SO₃⁻].</td>
</tr>
<tr>
<td>M-19, ES-MS</td>
<td>ES-MS m/z (%): 527, [M⁺Na⁺]; 522, [M⁺Na⁺]; 505, [M⁺Na⁺]; 473, [M⁺H⁺CH₂OH⁺]; 380, [M⁺H⁺NH⁺CH₂CH₂SO₃⁺]; 348, [M⁺H⁺CH₂OH⁺].</td>
<td></td>
</tr>
<tr>
<td>M-20, ES-MS</td>
<td>ES-MS m/z (%): 527, [M⁺Na⁺]; 505, [M⁺H⁺CH₂OH⁺].</td>
<td></td>
</tr>
<tr>
<td>M-21, ES-MS</td>
<td>ES-MS m/z (%): 537, [M⁺Na⁺]; 511, [M⁺H⁺].</td>
<td></td>
</tr>
<tr>
<td>M-22, ES-MS</td>
<td>ES-MS m/z (%): 537, [M⁺Na⁺]; 444, [M⁺H⁺].</td>
<td></td>
</tr>
<tr>
<td>M-23, ES-MS</td>
<td>ES-MS m/z (%): 500 (100) [M⁺H⁺]; 384 (100) [M⁺H⁺]; 340 (15) [M⁺H⁺CO⁺]; 310 (77) [M⁺H⁺CHO⁻].</td>
<td></td>
</tr>
<tr>
<td>M-24, ES-MS</td>
<td>ES-MS m/z (%): 431 (s) [2H⁺CH₂O⁻]; 2.05 (m, 2H⁻H₂); 2.21 (m, 2H⁻H₂); 5.36 (dd, IH, J₆, J₇ = 15.0 Hz) H-4; 6.04 (dt, IH, J₅, J₆ = 15 Hertz; 7.10–7.14 (4 MHz) aromatic H's; ppm.</td>
<td></td>
</tr>
<tr>
<td>M-25, ES-MS</td>
<td>ES-MS m/z (%): 537, [M⁺Na⁺]; 511, [M⁺H⁺].</td>
<td></td>
</tr>
<tr>
<td>M-26, ES-MS</td>
<td>ES-MS m/z (%): 537, [M⁺Na⁺]; 511, [M⁺H⁺].</td>
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<tr>
<td>M-27, ES-MS</td>
<td>ES-MS m/z (%): 409 (2H⁺)CH₂O⁻; 3.16 (s, 3H) CH₃OH; 2.09 (m, 2H⁻H₂); 2.11–1.5 (4 MHz) aromatic H's; 3.93 (m, 1H) H-5; 5.34 (dd, IH, J₆, J₇ = 16.2 Hz; 6.23 (dd, IH, J₅, J₆ = 15 Hertz; 7.10–7.14 (4 MHz) aromatic H's; ppm.</td>
<td></td>
</tr>
<tr>
<td>M-28, ES-MS</td>
<td>ES-MS m/z (%): 435, [M⁺H⁺].</td>
<td></td>
</tr>
<tr>
<td>M-29, ES-MS</td>
<td>ES-MS m/z (%): 399, [M⁺H⁺].</td>
<td></td>
</tr>
<tr>
<td>M-30, ES-MS</td>
<td>ES-MS m/z (%): 400, [M⁺H⁺].</td>
<td></td>
</tr>
<tr>
<td>M-31, ES-MS</td>
<td>ES-MS m/z (%): 416, [M⁺H⁺].</td>
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* For structural formulae, see schemes 3 and 4.
* For structures of fragments A–H, see figure 4.
* For structures of fragments I–M, see figure 5.
Separation of M-19 and M-20 was performed by HPLC with ammonium acetate/acetonitrile as eluent. The cyclization product was the taurine conjugate of metabolite M-15 is the taurine conjugate of metabolite M-21. In the FAB mass spectrum, a molecular weight of 385 Da was represented by the [M–H]− ion at m/z 384, and the [M+Na–2H]+ ion at m/z 406. The 1H NMR spectrum resembled the spectrum of M-15 but did not show the typical signals of the taurine moiety.

Metabolite M-25. The ESI mass spectrum indicated a molecular weight of 536 Da. The 1H NMR spectrum was identical in most details to that of metabolite M-28 (see below), with two additional 2H triplets at δ = 2.96 and 3.59 ppm, characteristic for the taurine moiety. Thus M-25 was identified as the taurine derivative of M-28.

Metabolite M-26. The molecular weight of 550 Da, as inferred from the ESI mass spectrum, was 14 Da higher than that of M-25, thereby suggesting an intact methyl ether moiety. The 1H NMR spectrum confirmed this interpretation. It exhibited all signals of the 1H NMR spectrum of metabolite M-27 (see below) and, as expected for a taurine derivative, two additional 2H triplets at δ = 2.96 and 3.59 ppm.

Metabolite M-27. In the ESI mass spectrum, the ions [M+Na]+ and [M+H]+ at m/z 466 and m/z 444, respectively, revealed a molecular weight of 443 Da. The 1H NMR spectrum was very similar to that of the drug cerivastatin but with some characteristic differences: the signal at δ = 3.75 ppm (H-3) had disappeared, and proton H-5 had shifted upfield (δ = 3.93 ppm instead of δ = 4.17 ppm). The signals of H-2 appeared nearly as a 2H triplet (δ = 2.09 ppm), indicating that two protons were attached at C-3. From these data, the structure of a C-3 reduction product was inferred.

Metabolite M-28. In the ESI mass spectrum, the molecular weight of 429 Da was indicated by the [M+H]+ ion at m/z 430. The 1H NMR spectrum showed only two differences in comparison with that of metabolite M-27. The signal of the methoxy group was lacking and the signal of the -CH2O- group had shifted downfield (δ = 4.32 ppm), as observed in the 1H NMR spectrum of the demethylated metabolite M-1. M-28 was the desmethyl derivative of M-27. This finding was confirmed by incubation of M-27 with mouse liver microsomes, yielding M-28 as single reaction product (data not shown).

Metabolite M-29. In the ESI mass spectrum, the molecular weight of 397 Da was indicated by the [M+H]+ ion at m/z 398. The 1H NMR spectrum was similar to that of metabolite M-19 but lacked the typical signals of the taurine moiety. Both spectroscopic findings are in agreement with a pentadienoic acid side chain.

Metabolite M-30. In the mass spectrum, the [M+H]+ ion at m/z 400 revealed a molecular weight of 399 Da, 2 Da higher than that of metabolite M-29. The 1H NMR spectrum showed that, compared with M-29, the olefinic signals of H-2 and H-3 had disappeared. Two additional 2H signals at δ = 2.08 ppm (H-2, t) and δ = 2.21 ppm (H-3, q) supported the proposed structure.

Metabolite M-32. The ESI mass spectrum provided a molecular weight of 415 Da due to the [M+H]+ ion at m/z 416. Instead of the two methylene groups (H-2, H-3) observed in the 1H NMR spectrum of M-30, the 1H NMR spectrum of M-32 showed a 2H multiplet at δ = 2.09 ppm and a 1H multiplet at δ = 4.32 ppm, characteristic for a −CH2CHOH− group. In accordance with that, the H-4 signal appeared as a doublet of doublets indicating an adjacent methine group. These findings are in agreement with the structure of a primary β-oxidation product of cerivastatin.

Discussion

Marked species differences exist both in pathways and extent of cerivastatin metabolism. The beagle dog shows the highest recovery of the parent drug and its lactone in plasma and excreta. In rats, unchanged cerivastatin predominates in plasma and liver at early time points and accounts for less than 10% of the dose in bile. In contrast, the drug is metabolized rapidly and almost completely by mice. Only traces are found unchanged in bile and urine. Similar species differences have been reported for fluvastatin (Tse et al., 1990) and pravastatin (Komai et al., 1992) and less pronounced also for lovastatin (Halpin et al., 1993).

Demethylation of the pyridyl-methyl ether forming metabolite M-1 is the only important phase I biotransformation observed in dogs and is also important in rats. In vitro investigations using human liver microsomes (Boberg et al., 1997) and subsequent in vivo studies in healthy volunteers (M. Rätdke, W. Mück, manuscript in preparation) have shown that this reaction is a main phase I biotransformation in man and is catalyzed by cytochrome P-450. In contrast, neither M-1 nor any derived secondary metabolites were detected in mouse plasma and excreta.

In dogs, both hydroxy groups, as well as the carboxylic acid function of the drug, are subject to phase II conjugation with glucuronic acid. To our knowledge, the three theoretically possible monoglucuronides of the dihydroxyheptanoic or dihydroxyheptenoic acid side chain common to all statin HMG-CoA reductase inhibitors have not been separated and spectroscopically characterized before. After demethylation to M-1, the new benzylic alcohol becomes the preferred site for conjugation reactions. Surprisingly, the glucoside M-5 is more prominent than the glucuronide M-4. Usually, in mammals, drug glucosidation represents a minor metabolic pathway if glucuronidation is possible. However, the M-1 glucoside has several features in common with other glucosides described in literature (Tang, 1990); it exhibits the β-configuration, is attached close to a system containing π-bonds, and is found in dogs.

For statin HMG-CoA reductase inhibitors, the interconversion between hydroxy acid and lactone form has been described to be reversible and rapid compared to other irreversible biotransformation reactions (Duggan and Vickers, 1990). The respective amounts balanced in the excreta may depend on sampling and sample handling.
conditions. Therefore unchanged cerivastatin and lactone metabolite M-8 can together be taken as a measure for the total unchanged drug present in the respective biological sample. Similarly, differentiation between lactone and acid form of primary metabolites like M-22 and M-1 is of limited value. The mechanism proposed for the lactonization reaction involves an intramolecular acylation by a coenzyme A thioester of the hydroxy acid (Duggan and Vickers, 1990).

The same thioester has been proposed to be a key intermediate in the β-oxidation of the dihydroxyheptanoic acid side chain of lovastatin, simvastatin, and pravastatin. Pentanoic acid, and—for pravastatin—propanoic acid, metabolites have been observed as products of one and two β-oxidation cycles, respectively (Duggan and Vickers, 1990; Vickers et al., 1990; Everett et al., 1991).

Mechanistically, a 3-hydroxypentanoic acid and a 2-pentenoic acid intermediate have been postulated for the first two-carbon degradation reaction (Vickers et al., 1990). However, for none of the other statin drugs have the carboxylic metabolites or their corresponding taurine conjugates been found in vivo as a consequence of these two metabolic steps. The cerivastatin metabolites described here provide unequivocal evidence that the postulated intermediates are involved in the side chain degradation and thereby confirm the proposed mechanism (scheme 4). Whereas the 3-hydroxypent-4-enoic acid M-32 and the 2,4-pentadienoic acid M-29 were isolated from in vitro hepatocyte incubations, the pent-4-enoic acids M-21 and M-30 were the main metabolites in rat plasma and liver and were also identified in mouse liver and/or plasma. The corresponding taurine conjugates M-16/M-19 and M-15/M-18 predominate in rat and mouse bile. A second β-oxidation cycle, leading to propenoic acid derivatives, was not observed.

In terms of β-oxidation, lovastatin and simvastatin show the same species selectivity as cerivastatin. For all three drugs, this pathway is exclusively important in the rodent species (Halpin et al., 1993; Vickers et al., 1990). After pravastatin and atorvastatin administration, these metabolites have additionally been identified in smaller amounts in dogs (Komai et al., 1992; Michniewicz et al., 1994), and in the case of fluvastatin, the pentanoic acid metabolite was detected in human plasma (Dain et al., 1993).

The reductive removal of the 3-hydroxy group in the cerivastatin molecule leading to metabolites M-25, M-26, M-27, and M-28 is found exclusively in mice. Moreover, this unique biotransformation has not been described for any of the other statin drugs.

Mechanistically, the discovery of these metabolites provides evidence for an additional ramification in the β-oxidation sequence (scheme 5). A similar ramification has been described for pravastatin at the state of the primary β-oxidation product (Komai et al., 1992). Propanoic acid metabolites have been found in addition to pentanoic acid metabolites. This indicates that the intermediate 3-hydroxy pentanoic acid CoA thioester (analogue to I in scheme 5) had two alternatives: it was either subject to a second β-oxidation cycle or to the elimination/reduction sequence. In the case of cerivastatin, this alternative exists for the CoA thioester of the drug itself, as outlined in scheme 5.

As has been discussed in detail for lovastatin (Halpin et al., 1993), an epimerization at C3 of the side chain is required before the β-hydroxypentanoyl thioester can enter the β-oxidation cycle. Similarly, the loss of a second two-carbon unit leading to propanoic acid metabolites requires epimerization at the former C-5 of the intact drug molecule. The finding that lovastatin and simvastatin differ from pravastatin in their ability to be metabolized down to propanoic acids indicates that the compounds differ in their ability to undergo this latter epimerization. The alternative reaction sequence leading to the saturated carboxylic acid derivatives has been interpreted as the result of the intermediate D-β-hydroxypentanoyl thioester entering the last steps of a fatty acid biosynthetic cycle (Halpin et al., 1993).

For cerivastatin, the different metabolite profiles observed in rats and mice appear to be the consequence of different alternative pathways open for the drug CoA ester. Cerivastatin readily epimerizes at C3 in both species, which finally leads to the metabolites shown on the left in scheme 5. However, only in mice did the cerivastatin thioester directly enter the fatty acid cycle, as shown on the right of scheme 5. The high amounts of metabolites M-27 and M-28 detected in mouse liver and plasma 30 min after drug administration indicate this to be a rapid biotransformation. Consequently, no demethylated drug M-1 and only traces of unchanged drug were detected in mice. As a further consequence, formation of the demethylated metabolite M-28 is described as a secondary reaction in scheme 3.

Almost all cerivastatin metabolites with modified dihydroxyheptanoic acid side chain are subject to conjugation with taurine. As has been observed previously in the case of lovastatin, taurine conjugation is not observed as long as a hydroxy group in β-position to the carboxylate group is present (Halpin et al., 1993). The cerivastatin metabolism provides further evidence that this might be a general steric and/or electronic feature required by the active site of the N-acyltransferases involved.

Chemical cyclization to tricyclic derivatives has only been observed with the fully conjugated 2,3,4,5-diunsaturated carboxylic acid derivatives M-16 and M-19. The influence of the electron-withdrawing substituent obviously facilitates the electrocyclic rearrangement. However, because the stereochemistry at the newly formed chiral
carbon atom in the products M-17 and M-20 has not been investigated, the reaction mechanism can not be discussed in detail.

The metabolites of cerivastatin formed in mice, rats, and dogs are derived from three basic metabolic pathways. Cytochrome P-450-mediated demethylation of the benzylic methyl ether is important in rats and dogs. On the other hand, $\beta$-oxidation of the side chain carboxylic acid occurs in rats and mice. The reductive transformation of a side chain $\beta$-hydroxy carboxylic acid into a saturated carboxylic acid is also observed in both rodent species, but at different positions in the metabolic cascade. In dogs, the drug and primary metabolites are subject to phase II conjugation with sugar-derived moieties, whereas the products of side chain degradation are conjugated with taurine in rats, as well as in mice.

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References


