IDENTIFICATION OF THE METABOLITES OF THE HIV-1 REVERSE TRANSCRIPTASE INHIBITOR DELAVIRDINE IN MONKEYS

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ABSTRACT:
Delavirdine mesylate (U-90152T) is a highly specific nonnucleoside HIV-1 reverse transcriptase inhibitor currently under development for the treatment of AIDS. The metabolism of delavirdine was investigated in male and female cynomolgus monkeys after oral administration of [14C-carboxamide]delavirdine mesylate at single doses of 80 mg/kg and multiple doses of 160 to 300 mg/kg/day. Desalkyl delavirdine was the major metabolite in circulation. In urine, desalkyl delavirdine accounted for nearly half of the radioactivity, with despyridinyl delavirdine and conjugates of desalkyl delavirdine accounting for most of the remaining radioactivity. Bile was mostly composed of desalkyl delavirdine and 6'-O-glucuronide delavirdine, with parent drug, 4-O-glucuronide delavirdine, and conjugates of desalkyl delavirdine as significant components.

In addition, several minor metabolites were observed in urine and bile of delavirdine treated monkeys. The metabolism of delavirdine in the monkey was extensive and involved N-desalkylation, hydroxylation at the C-4' and C-6' positions of the pyridine ring, hydroxylation at the C-4 position of the indole ring, pyridine ring cleavage, N-glucuronidation of the indole ring, and amide bond cleavage as determined by MS and/or one-dimensional and two-dimensional NMR spectroscopies. Phase II biotransformations included glucuronidation, sulfation, and β-N-acetylgalactosaminidation. The identification of the N-linked β-N-acetylgalactosamine and 4-O-glucuronide metabolites of delavirdine represents novel bio-transformation pathways.

Delavirdine mesylate (U-90152T, fig. 1), 1-[(5-methanesulfonamido-1-N-indol-2-yl)-carbonyl]-4-[3-[(1-methylethyl)amino]-pyridinyl]piperazine monomethanesulfonate, is a potent and selective inhibitor of the HIV-1 reverse transcriptase (1, 2), an enzyme which catalyzes the conversion of genomic viral RNA into proviral DNA. The process of reverse transcription is essential to HIV replication (3–5). Delavirdine is a member of the bis(heteroaryl)piperazine class of nonnucleoside reverse transcriptase inhibitors. Unlike nucleoside reverse transcriptase inhibitors such as 3'-azido-3'-deoxythymidine (AZT, zidovudine) (6, 7), 2'-azido-3'-deoxythymidine (ddT, didanosine) (8–10), 2'-3'-dideoxythystidine (ddC, zalcitabine) (11), 2',3'-dideoxy-3',5'-dideoxycytidine (d4T, stavudine) (12), and 2'-deoxy-2'-thiacytidine (3TC, lamivudine) (13, 14)–which need to be converted by cellular enzymes to the active deoxynucleotide triphosphates, delavirdine mesylate does not need activation for inhibition of reverse transcriptase.

Delavirdine has been shown to selectively inhibit recombinant HIV-1 reverse transcriptase with a median inhibitory concentration of 0.26 μM (2). The potent inhibition of replication by delavirdine was comparable to the antiviral activity of nucleoside or other nonnucleoside reverse transcriptase inhibitors (1, 2). Unlike nucleoside reverse transcriptase inhibitors that exhibit serious toxicity (15, 16), delavirdine has low cellular toxicity, causing less than 8% reduction in peripheral blood lymphocyte viability at 100 μM, and exhibits no significant inhibition of cellular polymerases α and β, with a median inhibitory concentration of >440 μM (2). Delavirdine mesylate is currently under phase III clinical evaluations. In the course of the preclinical evaluation of delavirdine, its metabolism was investigated in male and female cynomolgus monkeys after single and multiple oral dose administration of [14C-carboxamide]delavirdine mesylate. The results from this study, including the identification of the metabolites of delavirdine, are presented.

Materials and Methods

Chemicals. All chemicals used in this study were of analytical grade. Solvents were Burdick & Jackson high purity grade (Burdick & Jackson, Muskegon, MI). Water was distilled and purified through a Milli-Q reagent.
water system (Millipore Corp., Bedford, MA), Ultima Gold (Packard Instrument Co., Meriden, CT) was used for liquid scintillation counting (LSC). Carbo-sorb E (Packard) and Permafluor E+ (Packard) were used for combustion of samples. Ultima-Flo M (Packard) was used as scintillant for flow-through detection. β-Glucuronidase (EC 3.2.1.31 from Helix pomatia, Type H-5), sulfatase (EC 3.1.6.1 from Aerobacter aerogenes, Type VI), and Trizima buffer (Tris-HCl 0.2 M, pH 7.4) were obtained from Sigma Chemical Company (St. Louis, MO). Methanol-d₄ (99.96% D) was purchased from Cambridge Isotope Laboratories (Cambridge, MA).

Delavirdine mesylate, [¹³C]-carboxamide]delavirdine mesylate, U-96183, U-102466, U-88703, and U-96364 were synthesized by Pharmacia & Upjohn, Inc. The specific activity of the radiolabeled test substance was 34.7 μCi/mg, with a radiochemical purity of >99% by HPLC. The chemical purity of the nonradiolabeled delavirdine mesylate was 97.6%. The structure and position of the [¹³C]-label are shown in fig. 1.

Animals. Cynomolgus monkeys were obtained from the Pharmacia & Upjohn primate colony. The body weight of the monkeys ranged from 4.4 to 6.2 kg. Monkeys were housed in individual stainless steel metabolism cages. Environmental conditions were maintained as follows: lights 12 hr on/12 hr off, relative humidity 40–60%, temperature 68–75°F, ventilation 18–20 room air changes per hr. Monkeys were provided with food (Certified Monkey Diet 5048, PMI Feeds Inc., St Louis, MO, supplemented with fresh fruit) and water ad libitum. Monkeys were fasted overnight prior to and 4 hr after radiolabeled dose administration. The animals in this study were cared for and used in accordance with The Guide for the Care and Use of Laboratory Animals, DHEW Publication (National Institutes of Health) 85–23, 1985 and with The Animal Welfare Act Regulation 9 CFR 3, August 15, 1989 and as modified on March 19, 1991.

Institutional Use. A Packard Tri-Carb Liquid Scintillation Analyzer Model 1900 CA or Model 1901TR (Packard Instrument Co.) was used for radiactivity counting. A TurboVap LV evaporator (Zymark Corporation, Hopkinton, MA) or a SpeedVac concentrator Model SVE 100H or Model AS260 (Savant Instruments, Inc., Farmingdale, NY) was used to concentrate samples.

Chromatography. The HPLC system consisted of a Perkin Elmer Series 410 quaternary pump (Perkin-Elmer Corp., Norwalk, CT), a Perkin Elmer ISS 200 LC sample processor, a Perkin Elmer LC-235C diode array detector, and a Radiochromatic A-525 flow-through detector (Packard Instrument Co., Meriden, CT) containing a 500-μl liquid cell. A Foxxy 200 fraction collector (Isco, Inc., Lincoln, NE) equipped with a diverter valve was used to collect fractions at 0.25- to 1 min intervals on a recycling mode.

Solid phase extraction (SPE) was performed using Bakerbond octadecyl extraction columns (J.T. Baker, Inc., Phillipsburg, NJ) containing 500 mg of C₁₈ packing. Columns were washed with approximately 3 ml acetonitrile, then equilibrated with 6 ml 0.1 M ammonium acetate buffer (pH 4) before use. A Supelco Visiprep vacuum manifold (Supelco, Inc., Bellefonte, PA) was used for SPE.

Mass Spectrometry. Particle beam-liquid chromatography-mass spectrometry (PB-LC-MS) was performed using a Finnigan 4021 quadrupole (Finnigan MAT, San Jose, CA) or a VG Autospec Q hybrid (Fisons, Manchester, UK) mass spectrometer equipped with a particle beam interface (Thermabeam, Extrel Corp., Pittsburgh, PA; interface built at Pharmacia & Upjohn, Inc.). The instrument was operated in the positive chemical ionization (CI) mode using ammonia as reagent gas. Electron energy was set at 70 eV. Interface temperatures were controlled by Vestec thermospray electronics (Vestec, Houston, TX). Data were acquired and reduced by a Vector II data system (Teknivent, Tyler, TX). Data were acquired and processed using Interactive Chemical Information System version 8.11 software. A tuning solution containing 1 mg/ml delavirdine mesylate was infused at a rate of 5 μl/min by a syringe pump into the mobile phase flow path via a T connector. Argon was used as collision gas at a pressure of 1 × 10⁻⁶ mbarr and collision energy of 50 eV. Samples were analyzed on a YMC 5-μm phenyl 4.6 mm i.d. × 25 cm column connected to a YMC 5-μm phenyl 4.6 mm i.d. × 2.3 cm guard cartridge. The mobile phase consisted of elution at 0.5 ml/min with a 25 min 20% A/80% D, 5-min linear gradient to 80% A/20% D, followed by 10 min 80% A/20% D where A = acetonitrile, D = 0.1 M ammonium acetate (pH 4).

Nuclear Magnetic Resonance Spectroscopy. ¹H NMR spectra were recorded at 500 MHz using a Bruker AMX-500 spectrometer (Bruker Instruments Inc., Billerica, MA). Data were processed on a Bruker X-32 computer using Bruker UXNMR software version 9309013. Samples were dissolved in approximately 300–500 μl methanol-d₄, in a 3 mm i.d. Teflon insert (WIL-MAD, Buena, NJ); the Teflon insert was placed in a 5 mm NMR tube (WILMAD, Buena, NJ). Spectra were recorded at a temperature of 300 K as free induction decays of 32K complex points. A resolution enhancing Gaussian window was applied to the free induction decays of selected samples to better resolve the couplings of the sugar resonances. The residual CHD₃OH pentent was used as reference at 3.30 ppm.

2D NMR experiments were recorded in the phase-sensitive mode using the Time Proportional Phase Increment method (17) to obtain quadrature detection in the second dimension. All experiments used presaturation of the large solvent hydroxyl at 4.82 ppm during a relaxation delay of 0.8–1.0 sec. Data tables were either 1K by 1K or 2K by 1K with 256 or 512 increments in F1 and 1K or 2K complex free induction decay data points in F2. ¹H–¹H double quantum filtered COSY spectra (18) used sinbel-squared window functions shifted by π/2 radians. All other 2D experiments used sinbell-squared window functions which were shifted by π/2 radians. The ROESY spectrum (19) used a 2,070 Hz continuous-wave spin-locking field applied for 200 msec at the frequency of the solvent hydroxyl peak. TOCSY spectra (20) used a 60 msec spin-lock. Proton-carbon HMOC (21) experiments used a delay of D2 = 1/(2JCH) = 3.2 msec and globally optimized alternating-phase rectangular pulses decoupling (22) of ¹C during acquisition. 2D spectra were referenced to the solvent at 3.30 (¹H) and 49.0 ppm (¹C).

Ultraviolet Spectrometry. UV spectra were obtained on a Perkin Elmer LC-235C diode array detector. Effluent was monitored at 295 and 255 nm, using a 5-nm bandwidth, 16-sec peak width, and a scanning rate of 1 scan every 5 sec. Peak purity information was obtained by comparing the absorbance of each and every defined wavelength in 5-nm increments of the UV spectrum taken on the leading edge with the corresponding absorbance of the spectrum taken on the trailing edge (23). This comparison was determined at approximately 20% peak height. A given peak was taken as homogeneous if the peak purity index was 1.5 or lower.

Dosing and Sample Collection. Delavirdine mesylate and [¹³C]-carboxamide]delavirdine mesylate were dissolved in 80% propylene glycol/20% water containing 3 μl methanesulfonic acid per ml to a final concentration of 150 mg/ml. In addition, a nonradiolabeled formulation was prepared at a concentration of 150 mg/ml. Two male and two female monkeys received single oral radiolabeled doses of approximately 80 mg/kg and 47 μCi/kg. One additional male and one female monkey were administered multiple oral nonradiolabeled doses of approximately 160 mg/kg/day given twice a day for 7 days (one morning dose and a second dose 8 hr later), followed on day 8 by a single oral radiolabeled dose of approximately 80 mg/kg and 47 μCi/kg. Doses were measured gravimetrically and administered by oral gavage using a dosing syringe attached to an intubation tube. Immediately after radiolabeled dose administration, monkeys received approximately 5 ml 0.01 N HCl to ensure complete transfer of the dosing formulation. Urine was collected from the monkeys over the time periods 0–12, 12–24, and at 24-hr intervals up to 168 hr. Nonradiolabeled urine was immediately analyzed or stored at −20°C until further analysis. Blood samples (3-ml aliquots, total blood withdrawn 33 ml for single-dose and 42 ml for multiple-dose studies) were
collected at scheduled times through the 48-hr period after the radiolabeled dose, stored on ice, and centrifuged to separate the plasma.

Terminal bile was obtained at the time of sacrifice, 10–13 hr after the last dose, from a male monkey given 240 mg/kg/day and a female monkey administered 300 mg/kg/day doses of delavirdine mesylate (doses given three times a day) from a 3-month toxicity study.

Sample Preparation and Total Radiocarbon Analysis. Aliquots of urine containing 500 μl (3 replicates) and 100 μl portions of plasma (2 replicates) were measured gravimetrically, mixed with 5 ml of Ultima Gold, and analyzed by LSC. Radioactivity was measured by LSC with 1–10 min counting. Count rates in cpm were corrected to dpm using quench curves generated from sealed quenched standards in either Ultima Gold cocktail. The limit of detection was ~40 dpm (2x background).

HPLC Profiles of Plasma and Urine Samples. Plasma and urine samples were profiled for parent drug and metabolites by HPLC with flow-through radiochemical detection. Chromatographic analyses were performed on a YMC 5μ-basic 4.6 mm i.d. × 25 cm column using gradient elution at 1.0 ml/min with 10% A/90% D for 5 min, followed by a 35-min linear gradient to 60% A/40% D, then 60% A/40% D for 5 min (where A = acetonitrile, D = 0.1 M ammonium acetate buffer (pH 4)). The HPLC effluent was mixed with Ultima-Flo M in a 1:3 ratio. Peaks of radioactivity were quantified on the Radiomatic detector using peak area integration. Column recovery was determined by comparing the total radioactivity from the sum of the integrated peaks in the 14C chromatogram with total radioactivity. The radioactivity in a given chromatographic peak was expressed as a percentage of the total radioactivity eluted during the analysis. Values were not corrected for column recovery since essentially all of the injected radioactivity was recovered from the column. The amounts of delavirdine and its metabolites in urine were expressed as percentages of the radioactivity in urine.

A portion of plasma ranging in volume from 100 to 200 μl was mixed with 2 volumes of acetonitrile followed by centrifugation at 14,000 rpm for 5 min at 4°C. The supernatant was evaporated to dryness and reconstituted in 100 μl 10% acetonitrile/90% 0.1 M ammonium acetate buffer (pH 4). A 75-μl aliquot of prepared plasma sample was analyzed by HPLC with flow-through radiochemical detection.

Urine samples were centrifuged to remove large particulates. A 200-μl portion of urine samples collected over the time periods 0–12, 12–24, 24–48, and 48–72 hr was directly analyzed by HPLC with flow-through radiochemical detection using gradient elution (vide supra).

HPLC Profiles of Bile Samples. Bile samples were directly profiled for parent drug and metabolites by HPLC with UV detection at 295 nm. Chromatographic conditions were the same as described above. Peaks were quantified using peak area integration assuming the same molar absorptivity. Peaks were assigned as drug-related material based on chromatographic comparison of bile from delavirdine treated and control animals.

Metabolite Isolation. Metabolites were isolated from appropriate matrices as described below. In general, at each purification step an aliquot of each fraction was dissolved by lyophilization and isolated using reversed phase HPLC (YMC 5μ-a basic 4.6 mm i.d. × 25 cm, 25–35 min, 10% A/90% D, followed by 35–50% A/50% D, then 50% A/50% D for 5 min, followed by 60% A/40% D, then 60% A/40% D for 5 min (where A = acetonitrile, D = 0.1 M ammonium acetate buffer (pH 4)). After centrifugation at 14,000 rpm for 5 min at 4°C, the supernatants were combined, concentrated to dryness, and reconstituted in 225 μl 15% acetonitrile/90% 0.1 M ammonium acetate buffer (pH 4). An aliquot of the concentrated sample plasma was analyzed by LC-ESI-MS.

A 5-ml portion of urine collected over the time period 0–12 hr from a female monkey given a 80 mg/kg single-dose of [14C-carboxamide]delavirdine mesylate was concentrated to dryness and reconstituted in approximately 1.2 ml 15% acetonitrile/85% 0.1 M ammonium acetate buffer (pH 4). An aliquot of the concentrated urine sample was analyzed by LC-APCI-MS and LC-ESI-MS.

Terminal bile collected from a male monkey given 240 mg/kg/day doses of delavirdine mesylate for 3 months was directly analyzed by LC-ESI-MS. The purified MET-4a was dissolved in methanol for ESI-MS analysis. The purified MET-4b was dissolved in 300–400 μl of methanol-d4 for NMR analysis. A 50-μl aliquot of MET-4b in methanol-d4 was evaporated to dryness and reconstituted in 100 μl methanol for ESI-MS analysis. The purified MET-4c was dissolved in 500 μl methanol. A 20-μl aliquot was diluted with 180 μl 0.1 M ammonium acetate buffer (pH 4) for PB-LC-MS and ESI-MS analyses. The remaining sample was evaporated to dryness and processed as described above for NMR analysis. The purified MET-4d was dissolved in 100 μl methanol for ESI-MS analysis.

A portion of the purified MET-16 was dissolved in methanol for ESI-MS analysis. The remaining sample was dissolved in methanol-d4 as described above for NMR analysis.

Enzyme Hydrolysis. A 2.4-ml portion of urine collected 0–12 hr post-dose from a female monkey given a 80 mg/kg single-dose of [14C-carboxamide]delavirdine mesylate was lyophilized and the residue reconstituted in 2.4 ml of Trizma buffer. A 600-μl aliquot of the sample was mixed with 600 μl of sulfatase solution (2 units/ml in Trizma buffer) and incubated at 37°C for 1 hr. A 100-μl aliquot of the solution was analyzed by HPLC with radiochemical detection. The HPLC conditions consisted of 1.0 ml/min elution on a YMC 5μ-phenyl 4.6 mm i.d. × 25 cm column with 25–60% 15% A/85% D, 5–min linear gradient to 60% A/40% D, 10–min 60% A/40% D, 5–min 60% A/40% D, A = acetonitrile, D = 0.1 M ammonium acetate buffer (pH 4)). Samples were also analyzed on a YMC 5μ-basic 4.6 mm i.d. × 25 cm column eluted at 1.0 ml/min with 5–min 10% A/90% D, 5–min linear gradient to 60% A/40% D, 5–min 60% A/40% D. A control sample containing monkey urine and Trizma buffer was carried out similarly.

A 25-μl portion of terminal bile collected from a male monkey given 240 mg/kg/day doses of delavirdine mesylate in the 3-month toxicity study was mixed with 200 μl 0.1 M sodium acetate (pH 5) and 200 μl β-glucuronidase solution (45,500 units/ml in 0.2% saline). The mixture was incubated at 37°C for 1 hr. A 25-μl aliquot of the sample was analyzed by gradient HPLC with UV detection at 295 nm. A control sample was prepared by mixing 25 μl of bile with 200 μl 0.1 M sodium acetate (pH 5) and 200 μl 0.2% saline and analyzed similarly.
Results

Plasma Metabolite Profiles. A summary of the analyses of plasma samples for delavirdine and its metabolites by HPLC with radiochemical detection is presented in Table 1. Desalkyl delavirdine was the major radioactive component in circulation after single dose and in male monkeys after multiple dose, constituting 58% and 54% of the radioactivity in 0–8 hr plasma, respectively, with delavirdine accounting for 20% and 31%. In female monkeys given multiple doses, delavirdine and desalkyl delavirdine were the major radioactive components in circulation. The remaining radioactivity in plasma was associated with MET-4 and MET-6. A representative chromatogram is shown in fig. 2.

HPLC Profiles of Urine. Urine was analyzed for delavirdine and its metabolites by HPLC with flow-through radiochemical detection (Table 1). The metabolism of delavirdine was extensive in the monkey, with no detectable amount excreted in urine as intact drug. After single and multiple doses, the major component in urine was MET-5, accounting for 40–56% of the radioactivity in urine. In addition, 19–29% of the radioactivity in urine was excreted as MET-4 and 9–18% as MET-2. Several minor metabolites were also observed in urine, including MET-10, MET-3, and MET-6. A representative chromatogram is shown in fig. 2.

HPLC Profiles of Bile. Bile was analyzed for delavirdine and its metabolites by HPLC with UV detection. Extensive metabolism was observed in bile samples from both male and female monkeys. The major biliary components (% of drug-related material in bile) were desalkyl delavirdine (20–31%) and MET-6 (19–27%). Parent drug constituted 11%. Several minor metabolites were also observed in bile, including MET-2 (4%), MET-3 (7–11%), MET-4 (11–15%), MET-16 (5–9%), and MET-7 (4–8%). A representative chromatogram is shown in fig. 2.

Metabolite Isolation and Identification. The presence of desalkyl delavirdine in plasma, bile, and urine was indicated by HPLC retention time and UV spectra comparisons with a synthetic standard and was confirmed by LC-MS analysis. The ESI mass spectrum showed a pseudomolecular ion (MH⁺) for parent drug at m/z 457 (base peak) (fig. 3, table 2). Structurally informative fragmentation ions at m/z 378, 247, and 221 arise from loss of CH₃SO₂, cleavage of the carbonyl-indole linkage, and cleavage of the carbonyl-piperazine bond, respectively.

The major component in monkey plasma, bile, and urine, MET-5, had the same HPLC retention time and UV spectrum as a synthetic standard of desalkyl delavirdine (U-96183). The presence of this metabolite in plasma, bile, and urine was confirmed by LC-MS. The ESI mass spectrum of MET-5 showed a pseudomolecular ion at m/z 415, 42 amu lower than parent drug, indicative of loss of the isopropyl side chain (fig. 3, table 2). Loss of CH₃SO₂ gave an ion at m/z 336. Cleavage of the carbonyl-piperazine bond gave ions at m/z 179 and 237, while cleavage of the carbonyl-indole linkage resulted in a fragment at m/z 205. Further confirmation of the presence of desalkyl delavirdine in monkey urine was obtained by PB-LC-CI-MS.

MET-3 was present as a minor component in bile and urine of delavirdine-treated monkeys. Elution of MET-3 as a broad peak indicated the presence of more than one metabolite. MET-3 remained unchanged upon treatment of bile with β-glucuronidase (containing β-glucuronidase and sulfatase activities), suggesting that this metabo-

### Table 1

<table>
<thead>
<tr>
<th>Dose mg/kg/day</th>
<th>Sex/N</th>
<th>Dose Freq</th>
<th>Matrix</th>
<th>MET-2 Desphenpyril DLV</th>
<th>MET-10 Indole Carboxylic Acid</th>
<th>MET-3b</th>
<th>MET-4</th>
<th>MET-5 Desalkyl DLV</th>
<th>MET-6 6′-Gluc DLV</th>
<th>DLV</th>
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<tr>
<td>80</td>
<td>MF/4</td>
<td>Single</td>
<td>Plasma urine</td>
<td>ND</td>
<td>0.3 ± 0.3</td>
<td>4.7 ± 2.5</td>
<td>7.6 ± 5.5</td>
<td>57.9 ± 6.5</td>
<td>14.9 ± 8.2</td>
<td>19.5 ± 13.1</td>
</tr>
<tr>
<td>160</td>
<td>M/F</td>
<td>Multiple</td>
<td>Plasma urine</td>
<td>ND</td>
<td>9.1</td>
<td>14.9</td>
<td>28.5</td>
<td>40.3</td>
<td>4.0</td>
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<td>Plasma urine</td>
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<td>2.3</td>
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<td>55.4</td>
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<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
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</table>

**a** Mean ± SD in percentage of the radioactivity in plasma (0–8 hr for single-dose and multiple-dose male; 0–12 hr for multiple-dose female) and urine (0–72 hr). ND, not detected (peak area < 50 dpm).

**b** MET-3 was not identified.

**c** MET-4 represents a mixture of 4 metabolites: sulfate desalkyl delavirdine (MET-4a), 6′-sulfate desalkyl delavirdine (MET-4b), β-N-acetylgalactosamine desalkyl delavirdine (MET-4c), and 4′-sulfate desalkyl delavirdine (MET-4d).
olite was neither a β-glucuronic acid nor sulfate conjugate. MET-3 was unstable and on heating degraded to desalkyl delavirdine. This metabolite was not further characterized.

MET-2 was observed as a significant metabolite in urine and as a minor component in bile after administration of delavirdine mesylate. This metabolite had similar retention time as a synthetic standard of despyridinyl delavirdine (U-102466). Identification of MET-2 as despyridinyl delavirdine was established by HPLC co-injection with a synthetic standard and was confirmed by LC-MS. The ESI mass spectrum of MET-2 showed a pseudomolecular ion at \( m/z \) 323, indicating cleavage of the pyridine ring and an ion at \( m/z \) 237 resulting from cleavage of the carbonyl-piperazine linkage.

Another major metabolite in bile, MET-6, was also observed as a minor metabolite in plasma and urine of delavirdine-treated monkeys. Treatment of bile with β-glucuronidase (containing glucuronidase and sulfatase activities) resulted in the disappearance of MET-6 concomitant with an increase in MET-7. On further standing at room temperature within a few hours, MET-7 degraded to desalkyl delavirdine (MET-2). Upon treatment of monkey bile with sulfatase (containing no detectable β-glucuronidase activity), MET-6 remained unchanged. These data suggested that MET-6 was a glucuronic acid conjugate of MET-7.

Confirmation of MET-6 as a glucuronide was obtained by LC-MS analyses of monkey plasma, bile, and urine, and by LC-MS-MS of bile. The ESI mass spectrum of MET-6 showed a pseudomolecular ion at \( m/z \) 649, 192 amu higher than parent drug, indicative of addition of a hydroxy group followed by conjugation with glucuronic acid (fig. 3, table 2). Loss of the glucuronic acid moiety gave the base peak at \( m/z \) 473. Subsequent cleavage of the carbonyl-piperazine bond and the carbonyl-indole linkage gave ions at \( m/z \) 237 and 263, respectively, and indicated substitution on the right hand side of the molecule (i.e., piperazine ring, pyridine ring, or isopropyl group). Loss of the isopropyl moiety from the ion at \( m/z \) 263 to generate an ion at \( m/z \) 221 indicated that the substituent was located on the pyridine or piperazine rings. The position of substitution was determined to be at the pyridine ring C-6 by HPLC retention time comparison to MET-6 isolated from rat bile and identified by \(^1\)H NMR and MS (24).

Confirmation of MET-7 as 6'-hydroxy delavirdine was obtained by LC-MS analysis of monkey urine. The APCI and ESI mass spectra of MET-7 showed a pseudomolecular ion at \( m/z \) 473 (table 2), 16 amu higher than parent drug and indicative of the presence of a hydroxyl group. Cleavage of the carbonyl-indole linkage gave an ion at \( m/z \) 263, 16 amu higher than the corresponding ion for parent drug, and indicated substitution on the pyridine or piperazine rings. These data
together with the fact that enzyme hydrolysis of 6'-O-glucuronide delavirdine (MET-6) generated MET-7, identified MET-7 as 6'-hydroxy delavirdine.

MET-10 and MET-12 were observed as minor urinary metabolites using radiochemical and UV detectors, respectively. These metabolites eluted at the same HPLC retention times as the synthetic standards U-96364 (indole carboxylic acid metabolite) and U-88703 (N-isopropylpyridinepiperazine metabolite), respectively, arising from cleavage of the amide bond. Confirmation of the presence of the indole carboxylic acid metabolite in monkey urine was pursued by LC-MS. However, no spectroscopic information could be obtained because of the small amount present and to the insensitivity of the technique for this metabolite. On the other hand, ESI-MS was more sensitive for the N-isopropylpyridinepiperazine metabolite and confirmed a pseudomolecular ion at \( m/z \) 221 (table 2).

MET-4 was observed as a significant component in urine of delavirdine treated monkeys. Phenyl-phase chromatography indicated that four metabolites, MET-4a, MET-4b, MET-4c, and MET-4d, were present. Incubation of monkey urine with sulfatase resulted in the disappearance of MET-4b and MET-4d, concomitant with an increase in MET-15b and MET-15d, while MET-4a and MET-4d remained unchanged. These data indicated that MET-4b and MET-4c contained a phenolsulfate group, while MET-4a and MET-4c did not. To identify these metabolites, isolation from monkey urine was pursued.

MET-4a showed a UV \( \lambda_{\text{max}} \) at 296 nm, indicating the presence of an indole ring. Diode array analysis gave a purity index of 1.4 and indicated the absence of UV absorbing impurities. The \(^1\)H NMR (500 MHz, methanol-\(d_4\), fig. 5, table 3) spectrum showed the presence of six aromatic protons, eight piperazine protons, and a methylsulfonate group. Four aromatic resonances were similar to corresponding resonances in the parent drug and were assigned to protons on the indole ring (δ 7.55, 7.42, 7.17, and 6.83 ppm). A

**TABLE 2**

Summary of key mass spectrometry fragmentation for delavirdine and its metabolites

<table>
<thead>
<tr>
<th>Compound</th>
<th>Matrix</th>
<th>Ionization Mode</th>
<th>( \text{MH}^+ )</th>
<th>Fragments (m/z)( ^b )</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLV</td>
<td>Plasma, bile, urine</td>
<td>ESI 457</td>
<td>136(^c) 323 221</td>
<td>— 247(^d) 378(^d)</td>
</tr>
<tr>
<td>MET-2</td>
<td>Bile, urine</td>
<td>ESI 323</td>
<td>— — 237</td>
<td>— —</td>
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<td>— — 179(^c)</td>
<td>237 207 ( ^d) —</td>
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<td>ESI 511</td>
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<td>APCI 511</td>
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<td>ESI, APCI 618</td>
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<tr>
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<td>CI 618</td>
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<td>237(^d) 207 337(^d)</td>
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<td>95 323 179</td>
<td>— 207 337</td>
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<td>237 263(^d) 570(^c)</td>
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<td>237 221 353</td>
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<td>— 247(^f) 394(^f)</td>
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<td>ESI(^b) 633</td>
<td>— 499 221</td>
<td>— 247 553</td>
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\(^a\) APCI, atmospheric pressure chemical ionization; CI, chemical ionization; ESI, electrospray ionization.
\(^b\) Some fragments are not observed because of the structure of the compound or because of low concentrations.
\(^c\) Not observed in urine.
\(^d\) Not observed in bile.
\(^e\) Minus sulfate.
\(^f\) Minus N-acetylglucosamine.
\(^g\) Minus glucuronic acid.
\(^h\) Product-ion spectrum.
resonance for the pyridine H-6' was not observed and the remaining two aromatic resonances for the pyridine ring protons H-4' and H-5' at δ 7.13 and 6.91 ppm, respectively, were shifted upfield compared with corresponding resonances in the parent drug (δ 7.50 and 7.37 ppm). In addition, the H-4' and H-5' protons in MET-4b appeared as doublets with a coupling constant J4'-5' of 8.3 Hz, whereas in the parent drug these protons were observed as doublets of doublets with coupling constants J4'-5' of 8.3 Hz, J4'-6' of 1.4 Hz, and J5'-6' of 5.7 Hz. These data established that MET-4b was substituted at the pyridine ring C-6'. Assignments were confirmed by 2D COSY NMR (table 4), which established homonuclear spin coupling between H-7 and H-6, as well as between H-4' and H-5'. A 2D TOCSY NMR experiment further confirmed the assignments, with cross peaks between H-4 and H-7, H-7 and H-6, H-4 and H-6, and H-4' and H-5'. Additional resonances in the 1H NMR spectrum of MET-4b corresponded to the piperazine ring protons (δ 3.99 and 3.19 ppm) and the methylsulfonate group (δ 2.88 ppm). Resonances for the isopropyl side chain were not observed and indicated N-desalkylation had occurred. The 6'-substitution pattern was confirmed with a 2D heteronuclear correlation experiment (table 4), which showed four 13C aromatic resonances for the indole ring carbons (δ 121, 116, 106, and 113 ppm) and two resonances for the pyridine ring carbons at 121 and 111 ppm, corresponding to C-4' and C-5', respectively. The resonance for C-5' was shifted upfield relative to the corresponding resonance in the parent drug (δ 121 ppm), while C-4' remained unchanged. These spectroscopic data together with the enzyme hydrolysis results indicated a desalkyl delavirdine nucleus substituted at C-6' with a moiety containing an exchangeable proton, such as a sulfate group. Confirmation of MET-4b as 6'-sulfate desalkyl delavirdine was obtained by ESI and APCI mass spectrometries (fig. 4, table 2), which showed a pseudomolecular ion at m/z 511, 96 amu higher than desalkyl delavirdine and indicative of addition of a sulfate group. Loss of sulfate gave an ion at m/z 431, subsequent cleavage of the carbonyl-indole bond gave an ion at m/z 221. Cleavage of the carbonyl-piperazine linkage resulted in ions at m/z 275 and 237.

MET-4c showed a UV λmax at 300 nm and suggested the presence of an indole ring. The isolated MET-4c was extremely unstable and on concentration at room temperature hydrolyzed partially to give a radioactive peak with similar retention time as desalkyl delavirdine. These data suggested that MET-4c was a conjugate of desalkyl delavirdine.

The CI mass spectrum of MET-4c (table 2) showed a pseudomolecular ion at m/z 618, 203 amu higher than desalkyl delavirdine. The odd molecular weight (MW 617) for MET-4c indicated an odd num-

FIG. 4. Mass spectra of delavirdine and its metabolites from monkey urine.

Top left, APCI mass spectrum of MET-4a; bottom left, ESI mass spectrum of MET-4b; top right, APCI mass spectrum of MET-4c; bottom right, ESI mass spectrum of MET-4c.
the carbonyl-indole linkage resulted in an ion at m/z 415, and subsequent cleavage of the carbonyl-indole bond gave an ion at m/z 275, while cleavage of the carbonyl-indole linkage resulted in an ion at m/z 207. Cleavage of the piperezine-pyridine linkage gave ions at m/z 323 and 95. These spectroscopic data indicated that biotransformation had occurred on the amino group of the pyridine ring.

Although ions from m/z 522 to 618 observed by CI-MS represented <0.2% of the total ion current, the reconstructed mass chromatograms indicated that these ions were not noise signals. This was confirmed by ESI-MS, with a base peak at m/z 618 corresponding to the pseudomolecular ion and an ion at m/z 415 arising from cleavage of the sugar moiety. Likewise, the APCI mass spectrum of MET-4c showed a pseudomolecular ion at m/z 618 (fig. 4, table 2). Loss of water gave an ion at m/z 600. Loss of CH₃SO₂ and acetate gave an ion at m/z 498. Cleavage of the sugar gave an ion at m/z 415, and subsequent cleavage of the carbonyl-piperazine linkage gave ions at m/z 179 and 237. Loss of CH₃SO₂ from m/z 237 resulted in an ion at m/z 159. Exact mass measurements gave a molecular mass of 618.2359 (calc. 618.2346) and an empirical formula of C₂₅H₂₆N₅O₄S₂.

The ¹H NMR (500 MHz, methanol-d₄, fig. 5, table 3) spectrum of MET-4c was very complex because of the presence of unhydrolyzed MET-4c and desalkyl delavirdine and the acetylated sugar arising from hydrolysis of MET-4c. However, because of the twofold ratio of MET-4c relative to desalkyl delavirdine, the resonances corresponding to unhydrolyzed MET-4c could be discriminated from those of desalkyl delavirdine and the acetylated sugar. MET-4c showed the presence of seven aromatic protons, eight piperazine protons, a methylsulfonate group, an N-acetyl group, and protons corresponding to a sugar moiety. Four aromatic resonances were similar to corresponding resonances in the parent drug and were assigned to protons on the indole ring (δ 7.56, 7.43, 7.17, and 6.85 ppm). Three aromatic resonances corresponded to the pyridine ring protons H-4’, H-5’, and H-6’ (δ 7.17, 7.01, and 7.68 ppm, respectively). Assignments were confirmed with a 2D COSY NMR experiment (table 4), which established homonuclear spin coupling between H-5’ and H-6’, H-6 and H-7, and H-4’ and H-5’. Likewise, a 2D TOCSY NMR experiment showed cross peaks between H-4’ and H-6’, H-5’ and H-6’, H-4 and H-7, H-4 and H-6, H-6 and H-7, and H-4’ and H-5’. The remaining resonances in the aromatic region that integrated for approximately half of the protons for MET-4c were assigned to desalkyl delavirdine (table 3). Additional resonances in the ¹H NMR spectrum of MET-4c corresponded to the piperezine ring protons (δ 4.07 and 3.03 ppm), the methylsulfonate group (δ 2.89 ppm), the N-acetyl group (δ 2.01 ppm), and the sugar protons (δ 5.91, 4.55, 4.01, 3.87, 3.67, 3.55, and 3.44 ppm). Resonances for the isopropyl side chain were not observed and indicated N-desalkylation had occurred. A coupling constant between the anomeric H-1’ (δ 5.91 ppm) and H-2’ (δ 4.55 ppm) of 7.3 Hz confirmed the β-linkage of the sugar. Sugar resonances were assigned with 2D COSY (fig. 6) and 2D TOCSY NMR experiments. In addition, ¹H homonuclear decoupling experiments showed coupling constants J₂₋₃, J₃₋₄’, and J₄’₋₅’ of 9.3, 10 and 8.5 Hz, respectively, and together with a 2D NOESY experiment established axial conformations for H-2’, H-3’, H-4’, and H-5’ and identified MET-4c as β-N-acetylgulosamyl desalkyl delavirdine.

MET-4d showed a UV λₘₚₙ at 302 nm and suggested the presence of an indole ring. Sulfatase hydrolysis indicated that MET-4d contained a phenolsulfate group. The APCI and ESI mass spectra of MET-4d (fig. 4, table 2) confirmed this assignment with a pseudomolecular ion at m/z 511, 96 amu higher than desalkyl delavirdine and indicative of addition of a sulfate group. Loss of sulfate gave an ion at m/z 431, subsequent cleavage of the carbonyl-indole bond gave an ion at m/z 221. Cleavage of the carbonyl-piperezine linkage resulted in ions at m/z 275 and 237, while cleavage of the piperezine-pyridine linkage gave an ion at m/z 323. The m/z 323 ion was common to both desalkyl delavirdine and MET-4d, while the m/z 275 ion was 96 amu higher than the corresponding ion for desalkyl delavirdine and indicated the presence of a sulfate group on the pyridine ring. HPLC retention time comparison with a previously isolated human urinary metabolite finalized the structure of the monkey MET-4d as the 4’-sulfate conjugate of desalkyl delavirdine (26).

MET-15b and MET-15d were observed upon treatment of MET-4b and MET-4d with sulfatase. These data indicated that MET-15b and MET-15d were 6'-hydroxy and 4’-hydroxy desalkyl delavirdine, respectively. Assignments were confirmed by APCI-MS (table 2), which showed pseudomolecular ions for MET-15b and MET-15d at m/z 431, 16 amu higher than desalkyl delavirdine. Fragments arising from loss of CH₃SO₂ (m/z 353), cleavage of the carbonyl-piperezine linkage (m/z 195) and cleavage of the carbonyl-indole bond (m/z 221)

Fig. 5. ¹H NMR spectra (500 Mhz, methanol-d₄) of the aromatic region of delavirdine and its metabolites.

From top to bottom: delavirdine, MET-4b, MET-4c, and MET-16. Resonances for the unhydrolyzed MET-4c (bold) and MET-5 (normal) resulting from hydrolysis of MET-4c are included.
were 16 amu higher than corresponding ions for desalkyl delavirdine and established hydroxylation on the pyridine ring.

MET-16 was observed as a significant metabolite in monkey bile. Treatment of bile with \(\beta\)-glucuronidase (containing glucuronidase and sulfatase activities) resulted in a decrease in MET-16. Upon treatment of monkey bile with sulfatase (containing no detectable \(\beta\)-glucuronidase activity), MET-16 remained unchanged. These data suggested that MET-6 was a glucuronic acid conjugate. Analysis of MET-16 by ESI-MS-MS showed a pseudomolecular ion at \(m/z\) 649, 192 amu higher than parent drug, indicative of addition of a hydroxyl group followed by conjugation with glucuronic acid (fig. 3, table 2). Loss of the glucuronic acid moiety gave an ion at \(m/z\) 473. Subsequent loss of \(\text{CH}_3\text{SO}_2\) gave an ion at \(m/z\) 394. Cleavage of the carbonyl-piperazine bond and the carbonyl-indole linkage gave ions at \(m/z\) 221 and 247, respectively, and indicated substitution on the indole ring. To determine the position of substitution, MET-16 was isolated from monkey bile.

The purified MET-16 showed a UV \(\lambda_{\text{max}}\) at 296 nm, indicative of the presence of an indole ring. The \(^1\text{H NMR}\) (500 MHz, methanol-\(d_4\), fig. 5, table 3) spectrum showed the presence of six aromatic protons, eight piperazine protons, the isopropyl side chain, a methylsulfonate group, and a sugar moiety. One aromatic resonance was observed as a doublet of doublets with coupling constants \(J_{\text{6-4}}\) and \(J_{\text{6-5}}\) of 2.4 and 4.0 Hz, respectively, and was therefore assigned to the pyridine H-6' proton. A 2D COSY NMR experiment (table 4) showed homonuclear spin coupling between H-6' and the multiplet at 6.98 ppm, integrating for two protons, and assigned the resonance at 6.98 ppm to the pyridine H-4' and H-5' protons. The remaining three aromatic resonances corresponded to protons on the indole ring. The resonance at 7.35 ppm appeared as a doublet with \(J_{\text{3-7}}\) of 0.8 Hz and was assigned to the indole H-3 proton. The resonance at \(\delta\) 7.25 ppm was observed as a doublet of doublets with coupling constants \(J_{\text{7-6}}\) and \(J_{\text{3-7}}\) of 8.8 and 0.8 Hz, respectively, and was assigned to the indole H-7 proton. The remaining aromatic resonance at \(\delta\) 7.32 ppm corresponded to the indole H-6 proton and appeared as a doublet with \(J_{\text{6-7}}\) of 8.7 Hz. Assignments were confirmed with 2D COSY and 2D TOCSY NMR experiments. These spectroscopic data established that MET-16 was substituted at the indole C-4. A 2D ROESY NMR experiment confirmed this assignment, showing cross peaks between the indole H-3 proton and the anomeric sugar proton, and established
that these protons were in close steric proximity. The strong steric interaction between the indole H-3 proton and the O-glucuronic acid predicts that the electron cloud around H-3 becomes deformed resulting in a shift to lower field. The 2D ROESY NMR experiment also indicated that the sugar moiety was directed towards the pyridine ring and accounted for the upfield shifts observed for the pyridine ring protons relative to the parent drug. Additional resonances in the 1H NMR spectrum of MET-16 corresponded to the piperazine ring protons (δ 4.08 and 3.09 ppm), a methine proton (δ 3.62 ppm), a methylsulfonyl group (δ 2.93 ppm), and a sugar moiety (δ 4.86, 3.62, 3.59, 3.54, and 3.47). The methyl protons in the isopropyl chain centered at δ 1.26 ppm were observed as an apparent doublet of doublets with coupling constants of 1.7 and 6.3 Hz. However, further 1H NMR experiments at 300 and 400 MHz revealed that what appeared to be a doublet of doublets was actually two doublets separated by 1.7 Hz (0.003 ppm) whose chemical shifts migrated with frequency. These data indicated that the methyl protons in the isopropyl chain were nonequivalent, probably because of the steric proximity of the sugar. A coupling constant between the anemic H-1" (δ 4.86 ppm) and H-2" (δ 3.62 ppm) of 7.9 Hz confirmed the β-linkage of the sugar. Sugar resonances were assigned with a 2D COSY NMR experiment (table 4). In addition, the coupling constants Jα,β, J1β,α, and Jα,γ′′ of 8.8, 8.8 and 9.5 Hz, respectively, established axial conformations for H-2", H-3", H-4", and H-5" and identified MET-16 as 4-β-O-glucuronide delavirdine.

MET-17 was present as a minor metabolite in bile of monkeys.
treated with delavirdine mesylate. Analysis of bile by LC-ESI-MS showed a pseudomolecular ion for MET-17 at \( m/z \) 633, 176 amu higher than delavirdine and indicated addition of an N-linked glucuronic acid. The position of glucuronidation was ascertained by ESI-MS-MS (fig. 7). Loss of \( \text{CH}_3\text{SO}_2 \) gave an ion at \( m/z \) 553, while loss of glucuronic acid resulted in an ion at \( m/z \) 457. Cleavage of the piperazine-pyridine bond resulted in an ion at \( m/z \) 499, 176 amu higher than the corresponding fragment in the parent drug, indicating substitution of the piperazine, indole, or sulfonamide nitrogens. Cleavage of the carbonyl-piperazine and carbonyl-indole linkages gave ions at \( m/z \) 221 and 247, which were also observed in the parent drug, and indicated substitution of the indole or sulfonamide nitrogens. The ion at \( m/z \) 539 corresponded to loss of \( \text{CH}_3\text{SO}_2\text{NH} \), and together with the ions at \( m/z \) 221 and 247, established glucuronidation of the indole N-1 nitrogen.

### Discussion

Desalkyl delavirdine was the major drug-related material in circulation after single-dose administration and in male monkeys after multiple-dose administration, with parent drug accounting for most of the remaining radioactivity and conjugates of desalkyl delavirdine (MET-4) and 6'-O-glucuronide delavirdine (MET-6) as minor components.

Delavirdine was metabolized extensively by the monkey. Unchanged delavirdine was not detected in urine. Desalkyl delavirdine accounted for nearly half of the radioactivity in urine, with despyridinyl delavirdine (MET-2) and conjugates of desalkyl delavirdine (MET-4) accounting for most of the remaining radioactivity. In ad-

### TABLE 4

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<th>MET-4c</th>
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**Proton chemical shift \( \delta \) in ppm relative to methanol.

**COSY**, correlation spectroscopy; homonuclear spin coupling observed to the indicated resonance.

**HETCOR**, heteronuclear correlation; \( ^{13}\text{C} \) assignments in ppm relative to methanol.

**NO**, not observed with the acquisition parameters used in the experiment.

---

**FIG. 7.** Electrospray ionization product-ion mass spectrum of MET-17 from monkey bile.
dition, the indole carboxylic acid metabolite (MET-10), MET-3, and 6'-O-glucuronide delavirdine (MET-6) were detected as minor components in monkey urine. Bile was mostly composed of desalkyl delavirdine and 6'-O-glucuronide delavirdine, with parent drug, 4'-O-glucuronide delavirdine (MET-16), and conjugates of desalkyl delavirdine (MET-4) as significant components. In addition, several minor metabolites were observed in bile of delavirdine-treated monkeys.

The use of 1H NMR spectroscopy proved to be indispensable for the identification of metabolites, especially to ascertain the location of glucuronidation and sulfation. Comparison of the chemical shifts and the splitting of the aromatic signals between delavirdine and the isolated metabolites (see fig. 5) was extremely useful in assigning the structures of the metabolites, in view of the small amounts isolated, the instability of some of the metabolites, and in the absence of synthetic standards. Likewise, the use of tandem mass spectrometry facilitated metabolite identification, establishing substitution on either the pyridine or indole rings by comparison of the fragmentation pattern between delavirdine and its metabolites.

Figure 8 illustrates the scheme for the metabolism of delavirdine in the monkey. The metabolism of delavirdine in the monkey involves six pathways. First, N-desalkylation to desalkyl delavirdine (MET-5, U-96183), followed by conjugation with sulfate (MET-4a) or N-acetylglucosamine (MET-4c). Second, hydroxylation of desalkyl delavirdine at the pyridine ring C-4 (MET-15d) or C-6 (MET-15b) and subsequent conjugation with sulfate give MET-4d and MET-4b, respectively. Alternatively, hydroxylation of delavirdine at the pyridine ring C-6' position to 6'-hydroxy delavirdine (MET-7) and subsequent conjugation with glucuronic acid give 6'-O-glucuronide delavirdine (MET-6). Third, the pyridine ring in 6'-hydroxy delavirdine is cleaved to give despyridinyl delavirdine (MET-2, U-102466). Fourth, hydroxylation of delavirdine at the indole ring C-4 position and conjugation with glucuronic acid to 4'-O-glucuronide delavirdine (MET-16). Fifth, N-glucuronidation of delavirdine at the indole ring (MET-17). A sixth pathway, involves amide bond cleavage with release of the N-isopropylpyridinepiperazine (MET-12, U-88703) and the indole carboxylic acid (MET-10, U-96364) metabolites.

Of particular interest are the formation of the N-linked β-N-acetylglucosamine conjugate of desalkyl delavirdine, the 4'-O-glucuronide conjugate of delavirdine, and N-glucuronidation of the indole nitrogen. N-acetylglucosaminidation has been reported as a selective conjugation reaction for 7β-hydroxylated bile acids such as ursodeoxycholic acid (27–29), in humans. However, in the case of bile acids, conjugation with N-acetylglucosamine occurs via O-linkage. Hydroxylation of indoles has been reported at the 3-, 5-, 6-, and 7-positions (30–33). Indole 4-hydroxylation of 1-methyltetrahydrocarboline has been observed in vitro, but not in vivo (34). The formation of this metabolite was attributed to pretreatment of the rats from which hepatocytes were isolated with 3-methylcholanthrene (34), a potent inducer of certain forms of cytochrome P450. Likewise, the only precedence for glucuronidation of the indole nitrogen is found for the carbazole N-linked glucuronide identified in bile samples of rats treated with carvedilol (35). Identification of the N-linked β-N-acetylglucosamine and 4'-O-glucuronide metabolites of delavirdine represents novel biotransformation pathways for xenobiotics.

**Fig. 8.** Metabolic pathway of delavirdine in monkeys.
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References


