IN VITRO AND IN VIVO METABOLISM OF DESOGESTREL IN SEVERAL SPECIES


Departments of Toxicology and Drug Disposition (C.H.J.V., S.F.M.K., R.M.E.V.) and Analytical Chemistry for Development (G.N.W.), N.V. Organon

(Received March 30, 1998; accepted May 19, 1998)

This paper is available online at http://www.dmd.org

ABSTRACT:

The metabolism of desogestrel (13-ethyl-11-methylene-18,19-dinor-17a-pregn-4-en-20-yn-17-ol), an orally active progestogen, was studied in vivo after administration of single oral doses to rats and dogs and in vitro using rat, rabbit, dog, and human liver microsomes. Metabolites were isolated and identified by NMR and MS analysis. After oral administration of [3H]desogestrel to rats and dogs, desogestrel was extensively metabolized in both species. Radioactivity was predominantly eliminated in the feces. In rats, desogestrel was metabolized mainly at the C3-, C5-, C11-, and C15-positions. Both in vivo and in vitro, the majority of metabolites were 3α-hydroxy,4,5α-dihydro derivatives. Other main metabolic routes for desogestrel in rats were 15α-hydroxylation and epoxidation of the C11-methylene moiety. In addition to phase I metabolites, glucuronic acid and sulfate conjugates of desogestrel were observed in vivo. In dogs, desogestrel was mainly metabolized at the C3- and C17-positions. In contrast to the rat metabolites, metabolites isolated from dog urine and feces were mainly 3β-hydroxy,4,5α-dihydro derivatives. In most of the metabolites present in dog urine and feces, the five-membered D-ring was expanded to a six-membered D-ring, i.e., D-homoannulation to a 17A-keto-D-homo ring. D-Homo metabolites, which were major metabolites in plasma, urine, and feces of dogs, were not observed in vitro. In dog liver microsomes, the 3-keto metabolite of desogestrel was the major metabolite. Similarly to dog liver microsomes, rabbit and human liver microsomes mainly converted desogestrel to its 3-keto metabolite. Predominant positions for further hydroxylation of the 3-keto metabolite of desogestrel were the C6-position (6β-hydroxy) and the ethyl substituent at the C13-position, for both species.

Desogestrel (13-ethyl-11-methylene-18,19-dinor-17α-pregn-4-en-20-yn-17-ol) is an orally active progestogenic prodrug (Viinikka et al., 1979; Hasenack et al., 1986; Back et al., 1987). The main desogestrel metabolite in vivo, i.e., the 3-keto metabolite of desogestrel (etonogestrel) (fig. 1), exerts progestogenic activity that is much stronger than that of the parent compound (Viinikka et al., 1976). Viinikka (1979) and Madden et al. (1989, 1990) demonstrated the formation of the 3-keto metabolite of desogestrel by human liver and human intestinal mucosa. Desogestrel undergoes phase I metabolism, and sulfate and glucuronide conjugates (phase II metabolites) are also formed.

The present study was performed to supplement the understanding of the metabolic routes for desogestrel in vivo and in vitro. The metabolism of desogestrel was studied in vivo in female rats and dogs, species generally used in preclinical safety studies. For metabolite profiling, rats were dosed with 56 μg/kg [3H]desogestrel and dogs with 67 μg/kg [3H]desogestrel. Metabolite profiles in plasma, urine, and feces were determined using HPLC. For the isolation and identification of metabolites, rats were dosed with 106 mg/kg [3H]desogestrel and dogs with 9.6 mg/kg [3H]desogestrel.

Species-related differences in the metabolism of desogestrel were investigated by incubation of [3H]desogestrel with rat, rabbit, dog, and human liver microsomes. Metabolites were isolated from microsomal incubations and from urine and feces samples, using solid-phase extraction and HPLC. Identification of the metabolites was performed by NMR and MS.

The in vitro metabolic routes observed in incubations with human liver microsomes were used to predict the in vivo metabolic routes for desogestrel in humans. The in vitro-in vivo correlations obtained for rats and dogs in this study were taken into account to strengthen these extrapolations.

Materials and Methods

Chemicals. [16-3H]Desogestrel (radiochemical purity, ≥99%) and [16-3H]Etonogestrel (radiochemical purity, ≥96%) were prepared by the Organic Synthesis Section of the Department of Drug Metabolism and Kinetics of N.V. Organon (Oss, The Netherlands). Desogestrel and etonogestrel were synthesized by the Department of Process Chemistry of N.V. Organon. All other chemicals were obtained from local commercial sources and were of analytical grade.

In Vivo Studies in Rats and Dogs. Animals. Female Wistar rats (approximately 200 g, HSD/CPB-WU) were obtained from Harlan CPB (Zeist, The Netherlands). Each rat received standard pelleted food (diet RMH-B; Hope Farms B.V., Woerden, The Netherlands) ad libitum. Female beagle dogs (approximately 11 kg, HSD/HFR:DoBe, purebred) were obtained from Harlan France, Z.I. Malcourné (Gannat, France). Each dog received 0.3 kg/day of standard pelleted food (diet DB-2106; Hope Farms B.V.). Food that had not been eaten after 24 hr was discarded.

Tap water was available ad libitum from drinking bottles and was refreshed each day. During sampling of urine and feces, the rats and dogs were housed individually in stainless steel metabolism cages, under standard conditions (temperature range, 20–26°C; relative humidity range, 43–87%).

Animal Treatments and Sampling. For determination of the extent of excretion of radioactivity in urine and feces and the metabolite profiles in plasma (dogs only), urine, and feces, three female rats and three female dogs were
treated with a single oral dose of 56 µg/kg (18.1 MBq/kg) or 67 µg/kg (1.2 MBq/kg) [3H]desogestrel, respectively. For the isolation and identification of metabolites, three female rats and one female dog received a single oral dose of 106 mg/kg (14.9 MBq/kg) or 9.6 mg/kg (2.3 MBq/kg) [3H]desogestrel, respectively.

Rats and dogs received desogestrel as a suspension in gelatin (0.5%, w/v) and mannitol (5%, w/v), by gavage. Urine and feces samples of rats and dogs were collected in 24-hr fractions for 168 hr after dosing. Urine samples were collected in chilled containers. From the dogs, blood samples (approximately 10 ml) were obtained from an exterior jugular vein at 0.5, 1, 2, 4, and 6 hr after administration of the radioactive dose. All samples were stored at −20°C until analysis.

In Vitro Incubation Studies with Liver Microsomes. Preparation of Liver Microsomes. Liver tissue was obtained from female Wistar rats, a female beagle dog, and a female New Zealand White rabbit (HSD/CPB:NZW; Harlan CPB). Human liver microsomes were prepared from surgical waste liver tissue (from a 32-year-old female patient) that was kindly donated by the Groningen Human Liver Group, University of Groningen (Groningen, The Netherlands).

Liver samples were homogenized, using a Potter-Elvehjem homogenizer at 0°C, in 50 mM Tris-HCl buffer (pH 7.4) containing 0.25 M sucrose. Microsomes were prepared by centrifugation (20 min, 100,000 N/kg; supernatant, 2 × 75 min, 1,000,000 N/kg). The microsomal pellet was resuspended in 100 mM potassium phosphate buffer (pH 7.4) containing 20% (v/v) glycerol. The microsomal suspensions were characterized by the determination of cytochrome P450 and protein concentrations. The concentrations of cytochrome P450 were calculated using the method of Lowry et al. (1951), using bovine serum albumin as the standard.

Analytical Incubations. Analytical microsomal incubations with [3H]desogestrel were performed for the analysis of metabolite profiles. Microsomes (cytochrome P450 concentration, 0.25 µM) were incubated at 37°C in 2 ml of 100 mM potassium phosphate buffer (pH 7.4) containing 3 mM MgCl2, 5 mM glucose-6-phosphate, 0.5 mM NADP+, 1.25 units/ml glucose-6-phosphate dehydrogenase, and 10 µM (31.5 kBq/ml) [3H]desogestrel. After a preincubation period of 2 min at 37°C, incubations were started by the addition of 100 mM potassium phosphate buffer (pH 7.4) containing 3 mM MgCl2, 5 mM glucose-6-phosphate, 1 mM NADP+, 1.5 units/ml glucose-6-phosphate dehydrogenase, and [3H]desogestrel. After a preincubation period of 5 min, the incubations were started by the addition of the test compound. Rat hepatic microsomes were incubated with 50 µM (43.8 kBq/ml) [3H]etonogestrel and rabbit hepatic microsomes with 50 µM (35.0 kBq/ml) [3H]desogestrel. Incubations were carried under a gentle stream of 95% O2/5% CO2. Incubations were stopped after 3 hr of incubation by freezing (solid CO2/ethanol, approximately −80°C).

Sample Analysis. Determination of Concentrations of Radioactivity. The concentrations of radioactivity in plasma (dogs only), urine, and incubation samples were determined by liquid scintillation counting with a TRI-CARB 2500 TR/T (Canberra Packard, Groningen, The Netherlands). The concentrations of radioactivity in feces were determined by combustion in a model 387 sample oxidizer (Canberra Packard), followed by liquid scintillation counting. Feces samples were homogenized with approximately 2 volumes of Milli-Q (Millipore BV, Etten-Leur, The Netherlands) water before combustion.

Determination of Metabolite Profiles. Pooled urine was concentrated in a Speed Vac concentrator (Dumee, The Netherlands), to an appropriate volume for HPLC analysis. Pooled feces homogenates were extracted twice with acetonitrile. Extracts were evaporated to dryness, and the residues were dissolved in methanol. After sample treatment, the urine and feces samples were analyzed by HPLC.

Dog plasma was pooled according to sampling time point. Pooled plasma was applied to Bakerbond C18, solid-phase extraction columns (J. T. Baker EV, Deventer, The Netherlands). Columns were washed with 0.1 M ammonium acetate buffer (pH 4.2) and eluted with methanol (solvent B). Elution was performed with a linear gradient of 0–90% (v/v) solvent B in 30 min, at 50°C. The flow rate was 1.7 ml/min.

HPLC Analysis of the microsomal incubation samples was performed using a NovaPak C18 (3.9 × 150 mm) column (Waters, Etten-Leur, The Netherlands) and a gradient of 0.1 M ammonium acetate buffer (pH 4.2) (solvent A) and methanol (solvent B). Elution was performed with a linear gradient of 25–40% solvent B in 5 min, followed by a gradient of 40–70% solvent B in 10 min and a gradient of 70–90% solvent B in 2 min, at 50°C. The flow rate was 1 ml/min.

HPLC analysis was performed with a HP1090 liquid chromatograph equipped with a HP1040 diode-array detector (Hewlett Packard, Germany) and a FluoroOne A525 on-line radioactivity detector (Canberra Packard). Samples were spiked with unlabeled etonogestrel as a reference for retention time (UV signal at 254 nm). Metabolite numbers were assigned on the basis of retention times. Metabolites from plasma (P), dog urine/feces (D), rat urine/feces (R), and microsomal incubations (M) were numbered independently.

Isolation of Metabolites from Urine, Feces, and Incubation Samples. Urine and Feces. Rat urine was centrifuged (5 min, 27,000 N/kg), and the supernatant was concentrated in a Speed Vac concentrator. After centrifugation, the urine pool was subjected to HPLC analysis.

Dog urine was centrifuged (4 min, 7000 N/kg) in a Hettich Rotanta/P centrifuge, and the supernatant was applied to a LiChropro RPH 8 solid-phase extraction column (Merck, Amsterdam, The Netherlands). The column was washed with 2 volumes of 0.01 M ammonium acetate buffer (pH 4.2) and eluted with methanol/Milli-Q water (80:20, v/v; flow rate, 5 ml/min). The effluent was collected in fractions. Fractions containing radioactivity were pooled, concentrated in a Speed Vac concentrator to an appropriate volume, and subjected to HPLC.

Feces homogenates from the rats and the dog were extracted with acetonitrile. The extracts were concentrated in a Speed Vac concentrator to an appropriate volume and subjected to HPLC analysis.

The chromatographic conditions used for HPLC analysis of rat and dog urine and feces were as described in HPLC Analysis of Metabolite Profiles for the plasma, urine, and feces samples. The HPLC effluent was collected in fractions. Fractions constituting each peak of radioactivity were pooled and dried in a Speed Vac concentrator. Residues were dissolved in methanol/Milli-Q water (1:2, v/v). After sonication for 5 min, the resulting fractions were each subjected to a second HPLC analysis using the chromatographic conditions described in HPLC Analysis of Metabolite Profiles for the microsomal incubation samples. The HPLC effluent was collected in fractions at the approximate retention times of the eluting peaks of radioactivity. Fractions...
constituting each peak of radioactivity were pooled and dried in a Speed Vac concentrator. The residues were further processed for NMR and MS analysis.

Preparative Incubation Samples. The preparative incubation mixtures were applied to a LiChroprep RP18 solid-phase extraction column (Merck, Germany). The column was washed with 2 volumes of 0.01 M ammonium acetate buffer (pH 4.2) and eluted with methanol/Milli-Q water (80:20, v/v) and methanol. The effluent was collected in fractions (flow rate, 5 ml/min).

Fractions containing radioactivity were pooled, concentrated in a Speed Vac concentrator to an appropriate volume, and subjected to HPLC. The chromatographic conditions used for HPLC analysis were as described in HPLC Analysis of Metabolite Profiles for the microsomal incubations. The HPLC effluent was collected in fractions. Fractions constituting each peak of radioactivity were pooled and dried in a Speed Vac concentrator. For compounds isolated from incubations with rat hepatic microsomes, residues were further processed for NMR analysis. For compounds isolated from incubations with rabbit hepatic microsomes, residues were taken up in methanol and further purified by a second HPLC analysis, using the chromatographic conditions described in HPLC Analysis of Metabolite Profiles for the plasma, urine, and feces samples. Some metabolites were further purified using a μ-Bondapak C18 column (7.8 × 300 mm) and a flow rate of 2.5 ml/min. The HPLC effluent was collected in fractions. Fractions constituting each peak of radioactivity were pooled and dried in a Speed Vac concentrator. Residues were further processed for NMR and MS analysis.

Identification of Metabolites. NMR Spectroscopy. The 1H NMR spectra were recorded at 400 or 600 MHz with a DRX400 or DRX600 spectrometer (Bruker Spectrospin AG, Fallanden, Switzerland), under standard conditions. The metabolites were dissolved in CD3OD. The CD3OD signal was used as a reference and was set at 3.30 ppm. The chemical shifts are given in ppm.

MS. Electron ionization mass spectra were recorded at 70-eV electron energy with a HP5989 mass spectrometer (Hewlett Packard, Palo Alto, CA), using a HP59980B particle beam interface. Sample introduction was performed by HPLC using a Prodigy ODS (5 μm, 2 × 250 mm) column (Phenomenex (Boster), Amstelveen, The Netherlands), and a gradient of Milli-Q water (solvent A) and acetonitrile (solvent B). Isocratic elution was performed with solvent B (40%) for 1.2 min, followed by a linear gradient of 40–100% (v/v) solvent B in 13.8 min. The flow rate was 0.4 ml/min. The ion source temperature was 250°C, and the desolation chamber temperature was 60°C.

Sample introduction was also performed by GC using a HP5890 Series II gas chromatograph. The ion source temperature of the mass spectrometer was set at 200°C. The injector temperature was 230°C, and the detector B temperature was 280°C. A DB1 (15 m × 0.25 mm; film thickness, 0.25 μm) column (Alltech, Breda, The Netherlands) was used. The GC temperature program was as follows: initial temperature, 100°C; initial time, 1 min; rate, 30°C/min; final temperature, 280°C; final time, 5 min; total run time, 12 min; flow rate, 2 ml/min; vacuum compensation, on.

Particle-beam chemical ionization spectra were recorded with the equipment described above, using methane as the reagent gas, at 200-eV electron energy. Ion spray spectra were recorded in positive- and negative-ion modes, using the ion spray interface of an API mass spectrometer. Flow injection analysis was performed with an effluent flow of 0.25 M ammonium acetate/methanol (30:70) at 50 μl/min. The API-100 mass spectrometer was operated at positive and negative ion spray voltages of approximately 5000 V and −4500 V, respectively, with a nebulizer gas flow of air at 10 bar (prepressure, approximately 4 bar) and a curtain gas flow of nitrogen at 8 (prepressure, approximately 3 bar). The orifice was maintained at 25 and −25 V, whereas the ring was maintained at 275 and −275 V. Mass spectra were recorded from approximately 100 amu to 1100 amu, with a step size of 0.1 amu and a dwell time of 0.3 msec.

Results

Excretion of Radioactivity in Urine and Feces. Data on the excretion of radioactivity after oral administration of [3H]desogestrel to rats and dogs are given in table 1. The total excretion of radioactivity 0–168 hr after a single oral dose of 56 μg/kg [3H]desogestrel to female Wistar rats was 90.3 ± 3.5%. The 0–168-hr excretion in the urine was 15.6 ± 3.9%, and that in the feces was 74.7 ± 1.4%. The total 0–168-hr recovery of radioactivity in urine and feces for dogs receiving a single oral treatment with 67 μg/kg [3H]desogestrel was 86.4 ± 3.3%, of which 20.9 ± 2.0% was excreted in urine and 65.5 ± 2.8% in the feces.

Metabolites of Desogestrel Present in Plasma, Urine, and Feces of Rats and Dogs. The plasma metabolite profile obtained for female beagle dogs treated orally with 67 μg/kg contained five compounds, i.e. P1–P5. P1 eluted at the retention time of the 3-keto metabolite of desogestrel and P4 at the retention time of desogestrol. On the basis of the retention times of the metabolites identified from dog urine and/or feces, P3 and P5 were tentatively identified as the 17a-keto-d-homo metabolite of desogestrel (D6) and the 3β-hydroxy,4,5α-dihydro, 17a-keto-d-homo metabolite of desogestrel (D3), respectively (see below).

Desogestrel was extensively metabolized after oral administration to rats and dogs; no desogestrel was observed in urine samples, and <6% of the radioactivity in the feces extracts was accounted for by desogestrol. The metabolites isolated from urine and feces from one dog were identified as D1–D6, and those from rats were identified as R1–R14. The compounds isolated and identified from urine and feces samples from rats and the dog are listed in table 2. Because the background levels were relatively high in urine and feces metabolite profiles, compounds were described as being major or minor on the basis of visual inspection of the peak heights. The metabolite profiles of rat and dog urine and feces are given in fig. 2. Major metabolites identified in rat urine were R1 and R3, and those in rat feces were R8, R9, R10, R13, and R14. The major metabolite identified in dog feces was D3. For the identification of the metabolites, see below.

Identification of the Metabolites Present in Urine and Feces of Rats and Dogs. The NMR and MS data of the metabolites isolated from urine and feces of rats and dogs are summarized in tables 3 and 4, respectively.

3-Keto-Δ4,11-spiro-oxiran,15α-hydroxy Metabolite of Desogestrel (R1). The signals at 3.11 and 2.66 ppm, together with the absence of the 3-β-hydroxy metabolite of desogestrel, suggested an 11-spiro-oxiran substituent.
TABLE 2

<table>
<thead>
<tr>
<th>Species</th>
<th>Urine</th>
<th>Feces</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats</td>
<td>R1*</td>
<td>R2</td>
</tr>
<tr>
<td></td>
<td>R4</td>
<td>R5</td>
</tr>
<tr>
<td></td>
<td>R7</td>
<td>R8</td>
</tr>
<tr>
<td></td>
<td>R10</td>
<td>R11</td>
</tr>
<tr>
<td></td>
<td>R13*</td>
<td>R14*</td>
</tr>
<tr>
<td>Dogs</td>
<td>D1</td>
<td>D2</td>
</tr>
<tr>
<td></td>
<td>D4*</td>
<td>D5*</td>
</tr>
</tbody>
</table>

1. 3-keto-D,11-spiro-oxiran,15a-hydroxy metabolite of desogestrel; 2. O-glucuronide of 15a-hydroxy,3-keto-D metabolite of desogestrel; 3. 2-hydroxy,3-keto,4,5-dihydro,17β-O-sulfate metabolite of desogestrel; 4. 3-keto,5α,11-spiro-oxiran,15a-hydroxy metabolite of desogestrel (with X possibly being an O-sulfate substituent); 5. 3β-O-sulfate,4,5β-dihydro,15a-hydroxy metabolite of desogestrel; 6. 2-hydroxy,3-keto,4,5α-dihydro,17β-hydroxy metabolite of desogestrel; 7. 3β-O-sulfate,4,5α-dihydro,15a-hydroxy metabolite of desogestrel; 8. 3α-O-sulfate,4,5α-dihydro,15α-hydroxy metabolite of desogestrel; 9. 3β-O-sulfate,4,5α-dihydro,15a-hydroxy metabolite of desogestrel; 10. 3-keto-D,15a-hydroxy metabolite of desogestrel; 11. 3α-O-sulfate,4,5α-dihydro,11-spiro-oxiran metabolite of desogestrel; 12. 3-keto,5α,15a-hydroxy metabolite of desogestrel; 13. 3α-O-sulfate,4,5α-dihydro metabolite of desogestrel; 14. 3α-hydroxy,4,5α-hydroxy,17α-keto-D-homo metabolite of desogestrel; 15. 3β-hydroxy,4,5α-dihydro,17α-keto-D-homo metabolite of desogestrel; 16. 16α- or 16β-hydroxy metabolite of desogestrel.

3β-Hydroxy,4,5α-dihydro,15α-hydroxy Metabolite of Desogestrel (R9). The presence of a broad multiplet at 3.56 ppm and the absence of the Δ4 or 3-keto-Δ4 moiety suggested a 3β-hydroxy group. The signal at 3.99 ppm indicated a 15α-hydroxy group.

3-Keto-Δ4,15α-hydroxy Metabolite of Desogestrel (R10). A small triplet at 5.85 ppm indicated a 3-keto-Δ4 moiety. A multiplet (double triplet) at 4.05 ppm indicated the presence of a 15α-hydroxy substituent.

3α-O-Sulfate,4,5α-dihydro,11-spiro-oxiran Metabolite of Desogestrel (R11) (with Unknown Stereochemistry at the Spiro-oxiran Moiety). The signals at 2.92 ppm and 2.59 ppm, together with the absence of the 11-methylene protons, indicated an 11-spiro-oxiran moiety. A narrow multiplet at 4.56 ppm and the absence of the Δ4 or 3-keto-Δ4 moiety indicated a 3α-O-sulfate group. The chemical shifts of the 2α- and 4α-protons suggested the 4,5α-dihydro conformation.

3-Keto,4,5α-dihydro,15α-hydroxy Metabolite of Desogestrel (R12). The absence of the Δ4 or 3-keto-Δ4 moiety and the chemical shifts of the 1β-, 1α-, 2α-, 2β-, and 4α-protons (2.27 ppm) indicated a 3-keto,4,5α-dihydro moiety. The signal at 4.01 ppm indicated a 15α-hydroxy group.

3α-O-Sulfate,4,5α-dihydro Metabolite of Desogestrel (R13). The absence of the Δ4 or 3-keto-Δ4 moiety and the presence of a narrow multiplet at 4.56 ppm indicated a 3α-O-sulfate group. The chemical shifts of the protons at C2 (2.08 and 1.52 ppm) and C4 (2.05 and 1.30 ppm) in the 1H-correlated spectroscopy spectrum suggested 3α-O-sulfate,4,5α-dihydro conformation.

3α-Hydroxy,4,5α-dihydro,15α-hydroxy Metabolite of Desogestrel (R14). A narrow multiplet at 4.02 ppm and the absence of the Δ4 or 3-keto-Δ4 moiety indicated a 3α-hydroxy,4,5α-dihydro conformation. The signal at 3.99 ppm indicated a 15α-hydroxy group.

3β-Hydroxy,4,5α-dihydro,6α-hydroxy,17α-keto-D-homo Metabolite of Desogestrel (D1). A broad multiplet at 3.53 ppm and the absence of the Δ4 or 3-keto-Δ4 moiety suggested a 3β-hydroxy substituent. The multiplet at 3.13 ppm suggested a 6α-hydroxy substituent. There was no singlet at approximately 3.0 ppm, indicating the absence of the 17-ethinyl substituent; a double triplet at 2.55 ppm showed the presence of an axial proton at C17 (17β-proton). Together with the presence of a triplet at 0.65 ppm (CH₃ of the ethyl substituent at C13), this indicates the formation of a 17α-keto-D-homo ring.

3-Keto-Δ4,15α-hydroxy Metabolite of Desogestrel (D2). Metabolite D2 is the same metabolite as R10 and M6a.

3β-Hydroxy,4,5α-dihydro,17α-keto-D-homo Metabolite of Desogestrel (D3). A broad multiplet at 3.53 ppm indicated the presence of an equatorial hydroxy group at C3. The signals of the A-ring protons in the 1H NMR spectra were compared with the signals of a reference compound (3β-hydroxy,4,5α-dihydro-desogestrel) to establish the positions of the 3-hydroxy and the 5-proton (results not shown). The data indicated that this metabolite is the 3β-hydroxy,4,5α-dihydro derivative. The presence of the 17β-proton and the absence of the 17-ethinyl signal indicated a 17α-keto-D-homo metabolite.

3α-Hydroxy,4,5β-dihydro,17α-keto-D-homo Metabolite of Desogestrel (D4). The multiplicities and positions of the 3-hydroxy groups in metabolites D3 and D4 were very similar. However, metabolites D3 and D4 showed differences in the shifts of the A-ring protons (protons at C1, C2, and C4). The differences in the chemical shifts of the A-ring protons are an indication that the 3-hydroxy group of metabolite D4 is in the α-position and the 5-proton in the β-position. The presence of the 17β-proton signal and the absence of the 17-ethinyl signal indicated a 17α-keto-D-homo metabolite.

17α-Keto-D-homo Metabolite of Desogestrel (D5). The presence of the 17β-proton signal and the absence of the 17-ethinyl signal suggested a 17α-keto-D-homo metabolite.
16α- or 16β-Hydroxy Metabolite of Desogestrel (D6). The presence of a CH-OH substituent was suggested by a double doublet at 4.27 ppm, which was connected to the protons at C15. This indicated the presence of an hydroxy substituent at C16.

Metabolites Present in Microsomal Incubations. Microsomal Activities. Rabbit hepatic microsomes were most active toward [3H]desogestrel, followed by human hepatic microsomes. [3H]Desogestrel was completely metabolized by rabbit and human hepatic microsomes within 10 and 30 min of incubation, respectively. Dog hepatic microsomes showed intermediate activity toward [3H]desogestrel, compared with the other species, and rat hepatic microsomes appeared to be least active; after 45 and 60 min of incubation, the percentages of [3H]desogestrel remaining in incubations with rat and dog hepatic microsomes were similar.

The compounds present in HPLC metabolite profiles for microsomal incubation samples are given in table 5. The metabolite profiles for incubations of desogestrel with rat, rabbit, dog, and human liver microsomes are given in fig. 3. The major metabolites of desogestrel formed after incubations with hepatic microsomes were the 3-keto metabolite of desogestrel (M12) (rabbit, dog, and human), M5a (rat), M5b (rabbit), M6a and/or M6b (dog), M14 (rabbit and dog), and M15 (rat). For the identification of the metabolites, see below.

Identification of the Metabolites Present in Microsomal Incubations. The NMR and MS data for the metabolites isolated from microsomal incubations are summarized in table 6.

3-Keto-D4,6β-hydroxy,13-hydroxyethyl Metabolite of Desogestrel (M3, Isolated from Rabbit Incubations). The presence of a 3α-hydro group at 3.54 ppm and the absence of the signals between 5.0 and 6.0 ppm suggested 3α-hydroxy,4.5α-dihydro conformation. The multiplicity of the 6α-proton present at 3.15 ppm indicated a 6α-hydroxy substituent.

3-Keto-D4,6β-hydroxy Metabolite of Desogestrel (M5b, Isolated from Rabbit Incubations). The small doublet at 5.92 ppm and a
TABLE 3

<table>
<thead>
<tr>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>R5</th>
<th>R6</th>
<th>R7</th>
<th>R8</th>
<th>R9</th>
<th>R10</th>
<th>R11</th>
<th>R12</th>
<th>R13</th>
<th>R14</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.87</td>
<td>4.66</td>
<td>5.79</td>
<td>5.83</td>
<td>4.87</td>
<td>4.47</td>
<td>4.66</td>
<td>5.79</td>
<td>5.83</td>
<td>4.87</td>
<td>4.66</td>
<td>5.79</td>
<td>5.83</td>
</tr>
<tr>
<td>NMR data (ppm)</td>
<td>4.87</td>
<td>4.66</td>
<td>5.79</td>
<td>5.83</td>
<td>4.87</td>
<td>4.47</td>
<td>4.66</td>
<td>5.79</td>
<td>5.83</td>
<td>4.87</td>
<td>4.66</td>
<td>5.79</td>
<td>5.83</td>
</tr>
<tr>
<td>b-H (2/a-b-OH)</td>
<td>4.48</td>
<td>4.47</td>
<td>5.85</td>
<td>2.05</td>
<td>1.12</td>
<td>2.18</td>
<td>0.96</td>
<td>1.12</td>
<td>2.12</td>
<td>2.05</td>
<td>2.57</td>
<td>2.30</td>
<td>2.05</td>
</tr>
<tr>
<td>2,-H (3-a-OH, 5-a-H)</td>
<td>4.02</td>
<td>3.56</td>
<td>4.56</td>
<td>4.56</td>
<td>4.02</td>
<td>3.56</td>
<td>4.56</td>
<td>4.56</td>
<td>4.02</td>
<td>3.56</td>
<td>4.56</td>
<td>4.56</td>
<td>4.02</td>
</tr>
<tr>
<td>4-H (3-keto-1-a-H)</td>
<td>2.18</td>
<td>0.96</td>
<td>1.12</td>
<td>2.12</td>
<td>2.05</td>
<td>2.57</td>
<td>2.30</td>
<td>2.05</td>
<td>2.08</td>
<td>1.75</td>
<td>4.56</td>
<td>4.56</td>
<td>4.56</td>
</tr>
<tr>
<td>4,-H</td>
<td>2.12</td>
<td>2.05</td>
<td>2.57</td>
<td>2.30</td>
<td>2.05</td>
<td>2.08</td>
<td>1.75</td>
<td>4.56</td>
<td>4.56</td>
<td>4.56</td>
<td>4.56</td>
<td>4.56</td>
<td>4.56</td>
</tr>
</tbody>
</table>

3-Keto-Δ4,15α-hydroxy Metabolite of Desogestrel (M6a, Isolated from Rat Incubations). Metabolite M6a was the same metabolite as D2 and R10.

3-Keto-Δ4,13-hydroxyethyl Metabolite of Desogestrel (M6b, Isolated from Rabbit Incubations). A small triplet at 5.85 ppm was indicative of the presence of the 3-keto-Δ4 moiety. The two multiplets at 3.96 and 3.69 ppm, together with the absence of a three-proton triplet at approximately 1.02 ppm, indicated hydroxylation of the ethyl substituent at C13 to CH3CH2OH.

3α-Hydroxy, 4,5α-dihydro, 15α-hydroxy Metabolite of Desogestrel (M10, Isolated from Rat Incubations). Metabolite M10 was the same metabolite as R14.

3-Keto Metabolite of Desogestrel (M12, Isolated from Rabbit Incubations). A small triplet at 5.88 ppm was indicative of the presence of the 3-keto-Δ4 moiety.

3β-Hydroxy, 4,5α-dihydro Metabolite of Desogestrel (M13, Isolated from Rat Incubations). The absence of the signals between 5.0 and 6.0 ppm and the presence of a broad multiplet at 3.55 ppm indicated 3β-hydroxy, 4,5α-dihydro conformation.

3-Keto, 4,5α-dihydro Metabolite of Desogestrel (M14, Isolated from Rat Incubations). The absence of the signals between 5.0 and 6.0 ppm and the presence of a multiplet at 2.58 ppm (1β-proton), a double triplet at 2.46 ppm (2β-proton), a multiplet at 2.28 ppm (2α-proton), and a multiplet at 1.12 ppm (1α-proton) indicated 3-keto, 4,5α-dihydro conformation.

3α-Hydroxy, 4,5α-dihydro Metabolite of Desogestrel (M15, Isolated from Rat Incubations). The absence of the signals between 5.0 and 6.0 ppm and the presence of a small multiplet at 4.02 ppm indicated the presence of a 3α-hydroxy substituent.

Differentiation between M5a and M5b and between M6a and M6b. Metabolite M5a (isolated from rat hepatic microsomes) was found to coelute with metabolite M5b (isolated from rabbit hepatic microsomes). The same was true for metabolites M6a (isolated from rat hepatic microsomes) and M6b (isolated from rabbit hepatic microsomes). The metabolites from dog and human incubation samples were tentatively identified based on comparisons of the retention times with those of metabolites isolated from rat and rabbit incubation samples. M5b was not observed in the HPLC metabolite profiles of dog hepatic microsomes. Whether metabolite M5 formed by human hepatic microsomes represents M5a or M5b was not possible to establish with certainty. Close inspection of the retention times of M5 in the profiles for rat and rabbit hepatic microsomes obtained using two different sets of HPLC conditions suggested that the main component of M5 formed by human microsomes might be the 3-keto-Δ4,6β-hydroxy metabolite of desogestrel (M5b). This possibility is supported by the finding of the 3-keto-Δ4,6β-hydroxy, 13-hydroxyethyl metabolite of desogestrel (M3) as a main metabolite in incubations with human hepatic microsomes.

M6 was also found to represent two compounds; M6a, identified as the 3-keto-Δ4,15α-hydroxy metabolite of desogestrel, was formed by rat hepatic microsomes and M6b, identified as the 3-keto-Δ4,13-hydroxyethyl metabolite of desogestrel, was formed by rabbit hepatic microsomes. Whether metabolite M6 formed by dog and human hepatic microsomes represents M6a or M6b was not possible to establish with certainty. After oral administration of [3H]desogestrel to female dogs in vivo, the 3-keto-Δ4,15α-hydroxy metabolite of desogestrel (M6a) was a metabolite present in feces. This suggests that, for dog hepatic microsomes, M6 presumably contains M6a as the main component. For human hepatic microsomes, the presence of...
the 3-keto-Δ4,6β-hydroxyl,13-hydroxyethyl metabolite of desogestrel (M3) indicates that the 3-keto-Δ4,13-hydroxyethyl metabolite of desogestrel is likely to be formed; however, the formation of M6a cannot be excluded as a possibility.

**Discussion**

**In Vivo Metabolism of Desogestrel in Rats and Dogs.** The results of the metabolism study with desogestrel in rats and dogs showed that desogestrel is extensively metabolized in both species. Radioactivity was predominantly eliminated in the feces. In rats, desogestrel was metabolized mainly at the C3, C5, C11, and C15 positions. An hydroxy group was introduced at the 15α-position of desogestrel, followed by conjugation with glucuronic acid. The 15α-hydroxy metabolites were observed in both urine and feces, whereas the glucuronide analogue was observed only in urine samples from the rats.

The methylene moiety at the C11-position was epoxidized to a spiro-oxiran analogue. The stereochemistry at this spiro-oxiran group could not be elucidated by NMR analysis. At the C3-position, 3β-hydroxy and 3α-hydroxy groups were observed in combination with the reduction of the Δ4-double bond to the 4,5α- or 4,5β-dihydro substituent. A 3-keto moiety was also formed in combination with the Δ4-double bond or 4,5α-dihydro group. The majority of metabolites isolated from rat urine or feces were 3α-hydroxy,4,5α-dihydro derivatives.

The feces samples contained 3-hydroxy metabolites conjugated with sulfonic acid. Conjugation with sulfonic acid was also observed at the C17-hydroxy group, yielding a metabolite that was excreted in the urine. An unstable metabolite, excreted in the feces, was tentatively identified as the 3-keto,5-X,15α-hydroxy metabolite of desogestrel. Because of the polarity of this compound in HPLC analysis, this X moiety is possibly an O-sulfate substituent. The sulfate substituent may easily be eliminated from the metabolite, resulting in a Δ4-double bond. A minor biotransformation route in rats was hydroxylation at the C2-position.

In dogs, desogestrel was mainly metabolized at the C3- and C17-positions. In most of the metabolites, the five-membered D-ring was expanded to a six-membered D-ring, i.e., D-homoanannulation to a 17A-keto-D-homo ring. D-Ring homoanannulation is also a known
metabolic route for ethinylestradiol after sc administration to rabbits (Abdel Aziz and Williams, 1969). Schmid et al. (1983) have established a possible mechanism for the D-homoannulation reaction. First, the electron-rich triple bond of the ethinyl group is oxidized, resulting in the formation of a high-energy intermediate. Second, this intermediate can rearrange, by D-ring enlargement to a more stable molecule, to a 17α-formyl-17α-keto-D-homosteroid. The 17α-formyl group is oxidized further and can decarboxylate spontaneously, resulting in the 17α-keto-D-homosteroid. Schmid et al. (1983) demonstrated that in at least one of the two oxidative reaction steps, probably the first, cytochrome P450 is involved.

In dogs, the 3-keto moiety was also formed in combination with the Δ4-double bond. The 3β-hydroxy and 3α-hydroxy substituents were present in combination with the reduced Δ4-double bond 4,5α- or 4,5β-dihydro), as was found for rats. However, in contrast to the rat metabolites (mainly 3α-hydroxy,4,5α-dihydro dihydro derivatives), metabolites isolated from dog urine or feces were mainly 3β-hydroxy,4,5α-dihydro dihydro derivatives.

Feces samples from the dogs also contained the 15α-hydroxy metabolite of desogestrel. No sulfonic acid or glucuronic acid conjugates were found. Minor biotransformation routes in dogs involved hydroxylation at C6 (6α-hydroxy) and C16 (16α/16β-hydroxy).

**In Vitro Metabolism of Desogestrel.** This study shows desogestrel to be extensively metabolized by rat, rabbit, dog, and human liver microsomes. Desogestrel was metabolized very rapidly to the 3-keto metabolite in rabbit, dog, and human liver microsomes, whereas rat liver microsomes were least active in the formation of the 3-keto metabolite of desogestrel.

The 3-keto metabolite of desogestrel was further metabolized very rapidly in rat liver microsomes, compared with other species, by
A-ring reduction to a 4,5α-dihydro derivative and to 3α- or 3β-hydroxy,4,5α-dihydro derivatives. The reduction of the 3-keto moiety was mainly to the 3α-hydroxy configuration, as was observed in vivo. Additional hydroxylated metabolites were also observed, with hydroxylation at C15 (15α-hydroxy) and C6 (6α-hydroxy). All metabolic routes of desogestrel observed in vitro were also observed in vivo, with the exception of hydroxylation at the 6α-position, which was observed in vitro but not in vivo.

In dog liver microsomes, the 3-keto metabolite of desogestrel was the major metabolite, followed by the 3-keto,15α-hydroxy metabolite of desogestrel, which was also isolated from dog urine. Metabolites with D-ring homoanulation and A-ring reduction were not observed in the incubations of desogestrel with dog liver microsomes.

D-Homo metabolites, which were major metabolites in plasma, urine, and feces of dogs, were not observed in vitro; this was unexpected because, as described above, cytochrome P450 is probably involved in the D-homoanulation reaction. It is possible that the responsible cytochrome P450 is expressed at a higher level in the intestine, compared with the liver, so that the D-homoanulation reaction is not observed in the liver. Further study is required to establish the possibility of the formation of the D-homo metabolites in dog intestine. The major metabolic routes observed in the in vitro study were also observed in the in vivo study in dogs.

Similarly to dog liver microsomes, rabbit and human liver microsomes mainly converted desogestrel to its 3-keto metabolite. The predominant positions for further hydroxylation of the 3-keto metabolite of desogestrel after incubation with rabbit and human liver microsomes were the C6-position (6β-hydroxy) and the ethyl substituent at the C13-position. 6β-Hydroxylation is a known metabolic route for steroids, e.g. testosterone, androstenedione, and progesterone, in human liver microsomes and is primarily catalyzed by cytochrome P450 3A4 (Waxman et al., 1988).

In human liver microsomes, A-ring reduction of the 3-keto metabolite of desogestrel was not observed. Early studies investigating the 3α-hydroxysteroid dehydrogenase enzyme in human liver (Iyer et al., 1992) showed no activity with 3-ketosteroids containing a Δ4-double bond. However, the 3α-hydroxysteroid dehydrogenase enzyme reduced 4,5α- and 4,5β-dihydri dihydro derivatives at similar rates. The Δ4-reductase responsible for the reduction of the 4/5-double bond is predominantly found in the cytosolic fractions of human liver (Ward and Back, 1993). Hence, the A-ring-reduced metabolites of the 3-keto metabolite of desogestrel were not present. As described above, the formation of a 15α-hydroxy metabolite has not been proven but cannot be excluded as a possibility. The metabolic routes for desogestrel found in rats, rabbits, dogs, and humans are given in fig. 4.

Rats and dogs showed similarities in the in vitro and in vivo metabolism of desogestrel. By extrapolation of the in vitro metabolic routes observed in incubations with human liver microsomes, it is likely that formation of the 3-keto moiety and hydroxylation at the

---

**TABLE 6**

<table>
<thead>
<tr>
<th>Metabolite Isolated from Microsomal Incubations</th>
<th>M3</th>
<th>M5a</th>
<th>M5b</th>
<th>M6a</th>
<th>M6b</th>
<th>M10</th>
<th>M12</th>
<th>M13</th>
<th>M14</th>
<th>M15</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMR data (ppm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3a-H (3β-OH,5α-H)</td>
<td>3.54</td>
<td>4.02</td>
<td>3.55</td>
<td>4.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3β-H (3α-OH,5α-H)</td>
<td>5.92</td>
<td>5.92</td>
<td>5.85</td>
<td>5.85</td>
<td>5.88</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-H (3-keto-)</td>
<td>3.15</td>
<td>4.31</td>
<td>4.31</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6α-H (6β-OH)</td>
<td>0.81</td>
<td>1.74</td>
<td>1.25</td>
<td>2.48</td>
<td>0.95</td>
<td>0.91</td>
<td>1.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15β-H (15α-OH)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17-Ethanol (C18-CH3)</td>
<td>2.95</td>
<td>2.96</td>
<td>2.93</td>
<td>2.96</td>
<td>2.93</td>
<td>2.96</td>
<td>2.92</td>
<td>2.92</td>
<td>2.92</td>
<td>2.92</td>
</tr>
<tr>
<td>18-CH2-OH</td>
<td>3.96/3.70</td>
<td>4.05/3.99</td>
<td>3.96/3.69</td>
<td>4.05/3.99</td>
<td>4.05/3.99</td>
<td>4.05/3.99</td>
<td>4.05/3.99</td>
<td>4.05/3.99</td>
<td>4.05/3.99</td>
<td>4.05/3.99</td>
</tr>
</tbody>
</table>

**Fig. 4. Metabolic routes of desogestrel.**

- A: 2-hydroxylation (in vivo); B: oxidation to a 3α-hydroxy, 3β-hydroxy, or 3-keto moiety (in vivo/in vitro)—the 3-hydroxy groups were further conjugated with sulfate (in vivo); C: reduction to 5α-H (in vivo/in vitro); D: oxidation to 3β-H or 3-X, with X possibly an OSO3H (in vivo); E: 15α-hydroxylation (in vivo/in vitro), further conjugated with glucuronic acid (in vivo); F: sulfation of 17-hydroxy (in vivo); and G: epoxidation of the methylene group (in vivo).

**TABLE 6**

<table>
<thead>
<tr>
<th>Metabolite Isolated from Microsomal Incubations</th>
<th>M3</th>
<th>M5a</th>
<th>M5b</th>
<th>M6a</th>
<th>M6b</th>
<th>M10</th>
<th>M12</th>
<th>M13</th>
<th>M14</th>
<th>M15</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMR data (ppm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3a-H (3β-OH,5α-H)</td>
<td>3.54</td>
<td>4.02</td>
<td>3.55</td>
<td>4.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3β-H (3α-OH,5α-H)</td>
<td>5.92</td>
<td>5.92</td>
<td>5.85</td>
<td>5.85</td>
<td>5.88</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-H (3-keto-)</td>
<td>3.15</td>
<td>4.31</td>
<td>4.31</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6α-H (6β-OH)</td>
<td>0.81</td>
<td>1.74</td>
<td>1.25</td>
<td>2.48</td>
<td>0.95</td>
<td>0.91</td>
<td>1.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15β-H (15α-OH)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17-Ethanol (C18-CH3)</td>
<td>2.95</td>
<td>2.96</td>
<td>2.93</td>
<td>2.96</td>
<td>2.93</td>
<td>2.96</td>
<td>2.92</td>
<td>2.92</td>
<td>2.92</td>
<td>2.92</td>
</tr>
<tr>
<td>18-CH2-OH</td>
<td>3.96/3.70</td>
<td>4.05/3.99</td>
<td>3.96/3.69</td>
<td>4.05/3.99</td>
<td>4.05/3.99</td>
<td>4.05/3.99</td>
<td>4.05/3.99</td>
<td>4.05/3.99</td>
<td>4.05/3.99</td>
<td>4.05/3.99</td>
</tr>
</tbody>
</table>

**Fig. 4. Metabolic routes of desogestrel.**

- A: 2-hydroxylation (in vivo); B: oxidation to a 3α-hydroxy, 3β-hydroxy, or 3-keto moiety (in vivo/in vitro)—the 3-hydroxy groups were further conjugated with sulfate (in vivo); C: reduction to 5α-H (in vivo/in vitro); D: oxidation to 3β-H or 3-X, with X possibly an OSO3H (in vivo); E: 15α-hydroxylation (in vivo/in vitro), further conjugated with glucuronic acid (in vivo); F: sulfation of 17-hydroxy (in vivo); and G: epoxidation of the methylene group (in vivo).

**Fig. 4. Metabolic routes of desogestrel.**

- A: 2-hydroxylation (in vivo); B: oxidation to a 3α-hydroxy, 3β-hydroxy, or 3-keto moiety (in vivo/in vitro)—the 3-hydroxy groups were further conjugated with sulfate (in vivo); C: reduction to 5α-H (in vivo/in vitro); D: oxidation to 3β-H or 3-X, with X possibly an OSO3H (in vivo); E: 15α-hydroxylation (in vivo/in vitro), further conjugated with glucuronic acid (in vivo); F: sulfation of 17-hydroxy (in vivo); and G: epoxidation of the methylene group (in vivo).
C6-position (6β-hydroxy) and at the ethyl substituent at the C13-position would occur in vivo. In addition, it is possible to find D-homo metabolites in human samples; Abdel Aziz and Williams (1970) isolated a D-homo metabolite of ethinylestradiol from human urine after oral administration of ethinylestradiol to human subjects. Viinikka (1979) identified 3-keto,4,5α-dihydro and 3α-hydroxy,4,5α-dihydro derivatives of desogestrel after incubations with human liver homogenates. In addition, major urinary metabolites of gestodene (also a 3-keto-Δ4-steroid) were the tetrahydro-reduced metabolites (Ward and Back, 1993); therefore, the A-ring-reduced metabolites of desogestrel may be formed in vivo in humans. Phase II metabolism is also likely to occur in vivo, because sulfate and glucuronide conjugates of desogestrel were identified by Madden et al. (1989), who studied the intestinal mucosa metabolism of desogestrel in vitro by using the Ussing chamber technique.

Various phase I metabolic reactions, such as carbon hydroxylations, D-homoannulation, and reduction of the 3-keto-Δ4 moiety, are observed in the metabolism of desogestrel in several species. Cytochrome P450 is known to catalyze a large number of phase I metabolic routes. A-Ring reduction of the 3-keto metabolite of desogestrel may be catalyzed by 5α- and/or 5β-reductase and 3α- and/or 3β-hydroxysteroid dehydrogenase. The identification of the enzymes responsible for the phase I metabolism of desogestrel will be an interesting subject for further investigations.

References