Pergamon

0960-0760(95)00161-1

J. Steroid Biochem. Molec. Biol. Vol. 55, No. 2, pp. 271–278, 1995

0960-0760/95 $9.50 + 0.00

Metabolism of Equilin Sulfate in the Dog

A. Chandrasekaran,* M. Osman, J. A. Scatina and S. F. Sisenwine

Drug Metabolism Division, Wyeth-Ayerst Research, CN8000, Princeton, NJ 08543, U.S.A.

The metabolism of equilin sulfate was determined in female dogs receiving 2.5 mg/kg of [^{3}H]equilin sulfate alone or in a preparation that contained all the components that are present in the conjugated equine estrogen product Premarin®. The pharmacokinetic parameters of total radioactivity indicated that the drug is rapidly absorbed and it has a moderate half-life in plasma. The total radioactivity in plasma following administration of [^{3}H]equilin sulfate as part of a mixture of conjugated equine estrogens had significantly lower peak concentration (C_{max}), a lower area under the curve (AUC), a longer terminal half-life ($t_{1/2}$) and a longer mean residence time (MRT) than when [^{3}H]equilin sulfate was given alone, indicating that the other components in the conjugated equine estrogen preparation altered the pharmacokinetics of equilin sulfate. An average of 26.7 ± 4.4% of the administered radioactive dose was excreted in urine of dogs receiving [^{3}H]equilin sulfate. Again, a significantly lower percentage (21.4 ± 6.3%, $P = 0.023$) was eliminated in urine of dogs receiving [^{3}H]equilin sulfate in the conjugated equine estrogen preparation, indicating that the absorption of equilin sulfate was perhaps altered by the other components in the conjugated equine estrogen preparation. Metabolite profiles of plasma and urine were similar. Equilin, equilenin, 17β-dihydroequilenin, 17β-dihydroequilin, 17α-dihydroequilenin and 17α-dihydroequilin were present in both matrices. 17β-Dihydroequilin and equilin were the two major chromatographic peaks in plasma samples. 17β-Dihydroequilenin and 17β-dihydroequilin were the major metabolites in urine. In conclusion, following oral administration of [^{3}H]equilin sulfate to dogs, the radioactivity is rapidly absorbed. The disposition of equilin sulfate is altered by the other components that are present in the conjugated equine estrogen preparation Premarin®. The reduction of the 17-keto group and aromatization of ring-B are the major metabolic pathways of equilin in the dog.

J. Steroid Biochem. Molec. Biol., Vol. 55, No. 2, pp. 271–278, 1995

INTRODUCTION

Equilin sulfate is a major component, constituting approximately 24% of the conjugated estrogens, in the equine estrogen preparation Premarin® [1, 2]. Premarin® brand conjugated equine estrogen is the most widely prescribed estrogen replacement therapy for post-menopausal women in the U.S. The equine estrogen preparation Premarin® contains a mixture of at least ten sulfated steroids in a well-defined ratio. Besides equilin sulfate, estrone sulfate, $\Delta^{8,9}$-dehydroestrone sulfate, equilenin sulfate, 17α-dihydroequilin sulfate, 17α-dihydroequilenin sulfate, 17α-estradiol sulfate, 17β-dihydroequilin sulfate, 17β-dihydroequilenin sulfate, and 17β-estradiol sulfate are the other known components of Premarin® brand conjugated estrogen product [2].

Equilin sulfate, as part of conjugated equine estrogens, has been used commercially for over 50 years. The biological activity of equilin sulfate as an estrogenic agent in rats and humans and its protective effects against osteoporosis, as well as its ability to modify plasma lipid concentrations in humans, are well known [3–5]. The metabolic fate of equilin sulfate in humans has recently been reported [6]. Following oral administration of equilin sulfate, it is metabolized to 17β-dihydroequilin, 17β-dihydroequilenin and equilenin. Information regarding the pharmacokinetics and metabolism of this potent conjugated estrogen in animal models of pharmacology and toxicology is not available. The only animal in which the metabolism of equilin has been investigated is the pregnant mare, the source of equilin [7]. Therefore, the objective of this study was to determine the metabolism of equilin sulfate in female dogs receiving [^{3}H]equilin sulfate alone and to compare these data to those following administration of [^{3}H]equilin sulfate in a conjugated estrogen preparation that contains all of the components that are present in the Premarin® brand conjugated estrogen product.

*Correspondence to A. Chandrasekaran.
Received 10 Apr. 1995; accepted 15 Jun. 1995.

MATERIALS AND METHODS

Chemicals and reagents

[^{3}H]Equilin sulfate sodium ([2,4-^{3}H]1,3,5[10], 7-estratetraen-3-ol-17-one-3-sulfate, Lot #2637-185) was obtained from New England Nuclear Research Products, Boston, MA. The radiochemical purity, as determined by HPLC, was >97% and the specific activity was 156.5 μCi/mg. Unlabeled equilin sulfate sodium (Batch 43) and an extract of pregnant mares' urine containing all of the estrogenic components of the conjugated estrogens preparation Premarin® were obtained from Chemical Development, Wyeth-Ayerst Research, Montreal, Quebec, Canada. All other chemicals and reagents were of analytical grade from commercial sources.

Animals

Six female beagle dogs, 9–13 kg body weight, from an in-house colony (Princeton, NJ), were used in this study. The dogs were housed individually in stainless steel metabolism cages for the duration of the study. The animals were allowed access to water and food *ad libitum*, except when food was withheld 16 h prior to and 4 h after dosing.

Dosing and sample collection

The dosing solutions were prepared by mixing [^{3}H]equilin sulfate and unlabeled equilin sulfate such that each dose contained approx. 200 μCi of radioactivity and 2.5 mg/kg of equilin sulfate alone or a conjugated estrogen preparation which contained 2.5 mg/kg of equilin sulfate in 10 ml of sterile water. The molar amount of radioactive plus non-radioactive equilin sulfate was the same in the two dose groups, i.e. equilin sulfate alone and in combination with the other components of the conjugated estrogen preparation Premarin®. The doses were administered orally via gastric intubation in a balanced cross-over design. There was a 2 week washout period between doses. Urine was collected daily for 7 days. During the first 72 h, urine samples were collected over dry ice. Blood samples were taken via the cephalic vein using heparinized tubes at 0, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 h post-dose. Plasma was obtained from blood by centrifugation at 2500 rpm for 15 min.

Radioassay of specimens

Aliquots of urine, plasma and HPLC fractions were assayed directly in 15 ml of scintillation fluid (Aquasol-2, New England Nuclear). Radioactivity was measured with a Packard Tricarb 4640 liquid scintillation spectrometer. Counting efficiency was determined by the external standardization technique.

Determination of tritiated water

For determining percent of tritiated water, two 200 μl aliquots of each plasma and urine sample were placed into scintillation vials. To one set was added 15 ml of Aquasol-2 followed by radioassay. The other set was evaporated under a stream of nitrogen, reconstituted in 200 μl of water and radioassayed in 15 ml of Aquasol-2. The difference between the two sets was considered to indicate "volatile" tritium, most likely due to tritiated water.

Metabolite profiles in plasma and urine

Metabolite profiles were determined from untreated and Glusulase® hydrolyzed samples by HPLC. Only the 0–24 h urine samples and plasma samples up to 6 h were used for metabolite profiles, since the radioactivity concentrations were too low in the remaining collections. The urine samples were centrifuged at 2500 rpm to remove all particulate matter. Proteins in 0.5 ml aliquots of plasma samples were precipitated with 0.5 ml of acetonitrile. The samples were then centrifuged at 2500 rpm for 15 min and the supernatants analyzed by HPLC. This extraction procedure gave almost quantitative recoveries of the radioactivity (parent compound and metabolites), indicating that the procedural losses were less than 5%. Additional plasma and urine samples were diluted with equal volumes of 0.2 M sodium acetate buffer (pH 4.6). Glusulase® was added and the samples were incubated at 37°C for 24 h. To distinguish between glucuronide and sulfate conjugates, D-saccharo-1,4-lactone was added (1 mg/1000 units of enzyme) immediately prior to the addition of Glusulase®. Following incubation, acetonitrile (1 ml) was added to the incubates and the mixture was stirred for 30 min and then centrifuged at 2500 rpm for 15 min.

Aliquots of supernatants from the above samples were analyzed by HPLC. The HPLC system consisted of an Alltech Spherisorb C6 column (5 μ; 250 × 4.6 mm i.d. or 150 × 4.6 mm i.d.), a Spectra-Physics Model SP8780 autosampler and a Waters Model 625 HPLC pump. Metabolites were eluted at a flow rate of 1 ml/min using a mobile phase that consisted of methanol, acetonitrile and 0.05 M potassium phosphate buffer (pH 3.0) in a ratio of 15:25:60. Detection was accomplished with an ESA-Coulochem II electrochemical detector and a Radiomatic radioisotope detector. The potentials for the detector were set at 0.75 V for guard cell, 0.35 V for electrode 1 and 0.70 V for electrode 2 and the signal from electrode 2 was recorded on a Spectra-physics integrator.

Confirmation of the structures of metabolites

The structures of the metabolites were confirmed by EI–GC–MS analysis of TMS derivatives of extracts of hydrolyzed urine samples. The retention times and mass spectra of the metabolites were compared to those of authentic reference standards of equilin, 17β-dihydroequilin, 17α-dihydroequilin, equilenin, 17β-dihydroequilenin and 17α-dihydroequilenin. The extraction was accomplished using 15 ml of diethyl

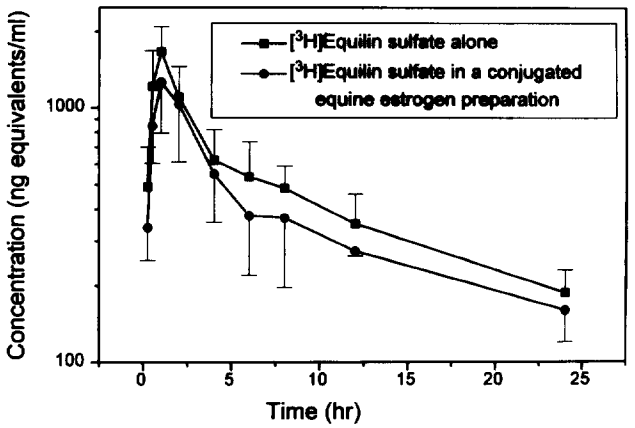

Fig. 1. Mean plasma concentrations of total radioactivity ([^{3}H]) in dogs receiving [^{3}H]equilin sulfate alone or in a conjugated equine estrogen preparation.

ether for 1 ml of urine. After evaporating the solvent, each extract or reference standard was reconstituted in 80 μl of toluene. BSTFA (10 μl) and pyridine (5 μl) were added and reacted at 65°C for 1 h to form TMS derivatives of the metabolites. The derivatized samples were analyzed using a Finnigan-MAT 8230 high resolution mass spectrometer directly interfaced to a Varian 3400 gas chromatograph. The source ionization mode was positive electron impact. The column used was a J&W DB-5MS 30 M × 0.32 mm i.d. The initial column temperature was 80°C with a 1 min hold programmed to 260°C at 10°C/min. The injection port temperature was 260°C. The injection volume was 2 μl. The data were acquired on an SS-300 data system.

Pharmacokinetic calculations

Concentrations of radioactivity in plasma are reported as ng equivalents of drug/ml. Peak plasma concentrations (C_{max}) and the time to peak (t_{max}) were determined by data inspection. Pharmacokinetic parameters were calculated from concentration vs time data by non-compartmental methods using the LAGRAN computer program [8]. The LAGRAN program employs the Lagrange method to calculate the area under the plasma concentration vs time curve (AUC) and area under the first moment curve ($AUMC$). The mean residence time (MRT) was calculated from the AUC and AUMC by the following formula [9]:

$$MRT = \frac{AUMC}{AUC}$$

The plasma terminal half-life of elimination ($t_{1/2}$) and the slope of the terminal phase (λ_z) were derived by log-linear regression analysis of the plasma radioactivity concentration vs time data. The half-life was calculated by dividing 0.693 by λ_z. The AUC was extrapolated to infinity by dividing the last measurable [^{3}H]concentration (C_p) by λ_z.

Statistical analysis

Statistical data analysis was performed by applying one-way analysis of variance for a randomized block design on the log of C_{max}, AUC_{0-24}, and $AUC_{0-\infty}$ and the reciprocal of t_{max}, $t_{1/2}$, MRT and percent urinary excretion. Pairwise comparisons using least significant difference method were performed for each parameter. Differences were considered statistically significant when P values were <0.02.

RESULTS

Pharmacokinetics

Mean plasma concentrations of total radioactivity ([^{3}H]) in female dogs following oral administration of 2.5 mg/kg of [^{3}H]equilin sulfate (as the sodium salt) alone or in a conjugated estrogen preparation which contained all the estrogens that are present in Premarin® are depicted in Fig.1. Mean pharmacokinetic values, from plasma concentration vs time data, are presented in Table 1. After oral administration of [^{3}H]equilin sulfate alone, the plasma t_{max} of total radioactivity was obtained within 1 h and the half-life was 16.3 ± 9.6 h for radioactivity.

When [^{3}H]equilin sulfate was administered as part of a mixture of conjugated equine estrogens as in the Premarin® brand conjugated estrogen preparation, total radioactivity concentrations exhibited lower C_{max}, AUC_{0-24}, $AUC_{0-\infty}$, a longer $t_{1/2}$ and a MRT than when [^{3}H]equilin sulfate was administered alone (Table 1). The differences in the C_{max}, AUC_{0-24} and $AUC_{0-\infty}$ were statistically significant ($P = <0.02$).

Urinary excretion of radioactivity

An average of 26.7 ± 4.4% of the administered radioactive dose was excreted in the urine of dogs receiving [^{3}H]equilin sulfate alone (Table 2). Most of the urinary radioactivity (21.8 ± 5.3% of the dose) was recovered in the first 24 h samples. A significantly lower percentage of the administered dose (21.4 ± 6.3%; $P = 0.023$) was eliminated in urine of

Table 1. Mean (±SD) pharmacokinetic parameters of total radioactivity in six female dogs following oral administration of either 2.5 mg/kg [^{3}H]equilin sulfate alone or in a mixture of conjugated equine estrogens

Pharmacokinetics parameters (units)	Dose	
	[^{3}H]equilin sulfate alone	[^{3}H]equilin sulfate in a conjugated equine estrogens mixture
C_{max} (μg equiv./ml)	1.67 ± 0.43	1.26 ± 0.46*
t_{max} (h)	1.0	1.0
AUC_{0-24} (μg equiv. h/ml)	10.8 ± 3.0	8.8 ± 3.2*
$AUC_{0-\infty}$ (μg equiv./h/ml)	15.1 ± 3.3	12.8 ± 2.6*
$t_{1/2}$ (h)	16.3 ± 9.6	19.1 ± 11.1
MRT (h)	20.8 ± 10.6	24.5 ± 13.7

*Significantly different from equilin sulfate alone ($P < 0.02$).

Table 2. Urinary excretion of radioactivity by six female dogs receiving 2.5 mg/kg of [^{3}H]equilin sulfate alone or in a preparation containing all the components that are present in the conjugated equine estrogen preparation Premarin®

Time period (h)	Percent of administered radioactivity	
	[^{3}H]equilin sulfate alone	[^{3}H]equilin sulfate in a conjugated estrogen preparation
0–24	21.8 ± 5.3	17.7 ± 7.1
24–48	3.5 ± 2.2	2.5 ± 1.4
0–48	25.4 ± 4.5	20.2 ± 6.2
0–168 (total)	26.7 ± 4.4	21.4 ± 6.3*

*Significantly different from equilin sulfate alone ($P < 0.02$).

dogs receiving [^{3}H]equilin sulfate in the conjugated equine estrogens mixture and the radioactivity recovered in the first 24 h was also lower (17.7 ± 7.1%).

Metabolite profiles

Urine. The loss of tritium as tritiated water in urine was less than 1% at any time point. Very little (< 5%) radioactivity in urine was extractable into diethyl ether indicating unconjugated metabolites represented only a small fraction of the total radioactivity. Direct injection of diluted urine on to HPLC revealed that most of the radioactivity was associated with polar conjugated metabolites.

HPLC–electrochemical chromatograms of enzyme (Glusulase®) hydrolyzed urine samples from dogs receiving [^{3}H]equilin sulfate contained six peaks (Fig.2). The retention times of the six peaks corresponded to those of 17β-dihydroequilenin (31.2 min), 17β-dihydroequilin (35.0 min), 17α-dihydroequilenin (37.3 min), 17α-dihydroequilin (43.6 min), equilenin (45.1 min) and equilin (48.6 min) in the HPLC–electrochemical chromatograms. Also, the HPLC–radiochromatograms of urine samples of dogs receiving [^{3}H]equilin sulfate alone were similar to those of the urine samples from dogs receiving [^{3}H]equilin sulfate in the conjugated estrogens preparation (Fig.3). Incubation of urine samples with Glusulase® in the presence of saccharolactone hydrolyzed only 20% of the conjugates, indicating that 80% of all these estrogens existed in the form of glucoronide conjugates. Results obtained from the quantitation of the radioactivity associated with each peak in the enzyme hydrolyzed 0–24 h urine samples from dogs receiving [^{3}H]equilin sulfate are given in Table 3. The major metabolites were determined to be 17β-dihydroequilin (31%) and 17β-dihydroequilenin (43%), while conjugates of equilin accounted for only about 6% of the 0–24 h urinary output.

Plasma. As in the urine samples, less than 1% of the radioactivity in the plasma was due to "volatile tritium" at all time points. Most of the radioactivity was due to polar conjugated metabolites, since > 95%

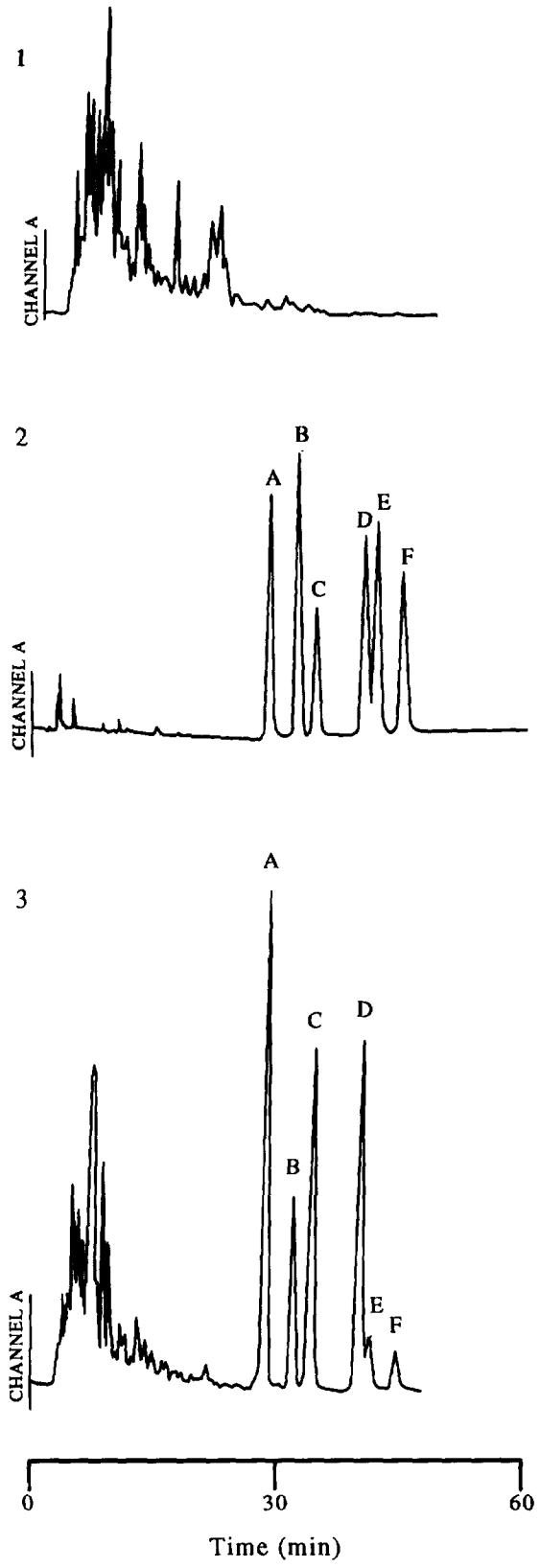

Fig. 2. HPLC–electrochemical chromatograms of: (1) hydrolyzed control urine sample; (2) reference standards, 17β-dihydroequilenin (A), 17β-dihydroequilin (B), 17α-dihydroequilenin (C), 17α-dihydroequilin (D), equilenin (E), equilin (F); and (3) hydrolyzed 0–24 h urine sample from a dog receiving 2.5 mg/kg of [^{3}H]equilin sulfate.

of the radioactivity was extractable into ether only after hydrolysis with enzyme. Plasma radioactivity profiles in enzyme-hydrolyzed samples were similar to those in the hydrolyzed urine samples. The radioactivity profiles were similar both in the plasma of the dogs receiving [^{3}H]equilin sulfate alone and the dogs receiving the conjugated estrogen preparation. HPLC–electrochemical chromatograms (Fig.4) of hydrolyzed plasma samples from dogs receiving [^{3}H]equilin sulfate showed six peaks that were not in the control samples. These peaks were identified as 17β-dihydroequilenin (12%), 17β-dihydroequilin (46%), 17α-dihydroequilenin (3%), 17α-dihydroequilin (7%), equilenin (4%) and equilin (28%) by comparing the retention times with those of the reference standards. 17β-Dihydroequilin (46%) and equilin (28%) were the major peaks compared to the others.

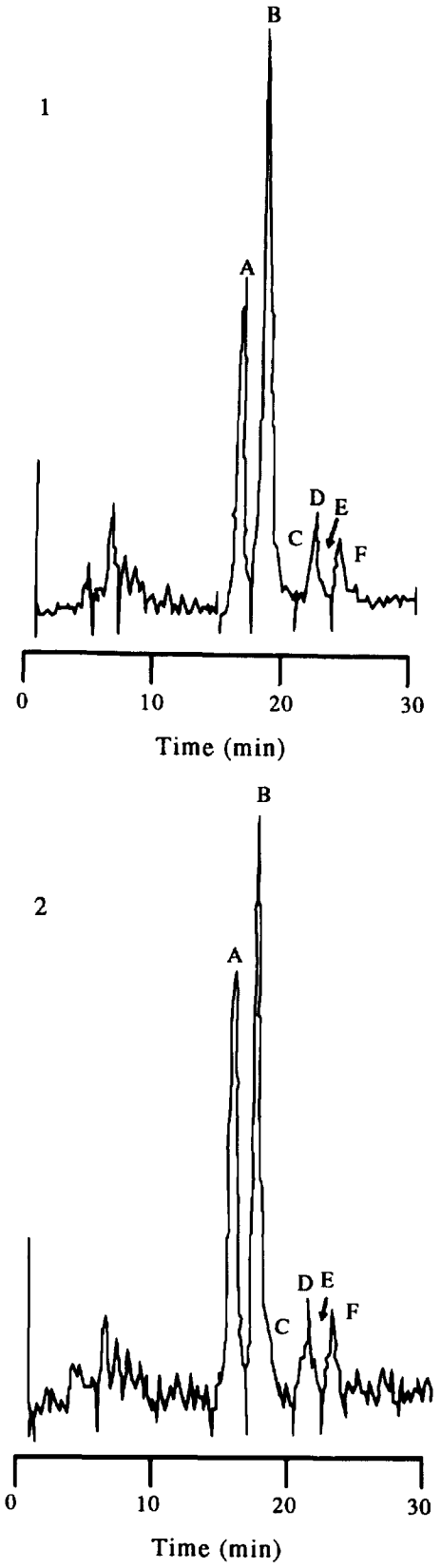

Fig. 3

Confirmation of metabolite structures

Structural confirmation of metabolites in urine was accomplished by co-chromatography of samples with reference standards and by EI–GC–MS. The reconstructed ion chromatograms of TMS derivatives of extracts of hydrolyzed urine showed six peaks that were not in control samples. The EI–GC–MS characteristics of these six peaks are summarized in Table 4. The mass spectrum of a peak with a retention time that was the same as that of the reference equilin standard showed a molecular ion at m/z 340 and other fragments that were identical to that of authentic equilin standard. Based on these data it was identified as equilin. Another peak was identified as equilenin based on the retention time and mass spectral fragmentation pattern that are characteristic for 17-ketonic estrogen with an aromatic ring-B structure [10]. Two peaks showed mass spectra with molecular ions at m/z 414. However, the intensity of the molecular ions were different for these two peaks. 17β- and 17α-dihydroequilin produced similar mass spectra as these two peaks. Also, 17α-dihydroequilin produced a weaker molecular ion than the 17β-isomer. Another set of metabolites also showed fragments that are characteristic of C-17 hydroxy isomers. The molecular ions for these isomers were two mass units lower than dihydroequilin. The structures of these two metabolites were confirmed as 17β- and 17α-dihydroequilenin, since the mass spectra were identical to those of the reference standards.

DISCUSSION

The metabolism of equilin sulfate was investigated in the dog following oral administration of [^{3}H]equilin

Fig. 3. HPLC–radio chromatograms of: (1) hydrolyzed 0–24 h urine sample from a dog receiving 2.5 mg/kg of [^{3}H]equilin sulfate; and (2) a hydrolyzed 0–24 h urine sample from a dog receiving 2.5 mg/kg of [^{3}H]equilin sulfate in a conjugated estrogen preparation. Retention times of peaks A–F correspond to those of 17β-dihydroequilenin (A), 17β-dihydroequilin (B), 17α-dihydroequilenin (C), 17α-dihydroequilin (D), equilenin (E), and equilin (F).

Table 3. Estimated average percentage of radioactivity associated with each compound in enzyme hydrolyzed 0–24 h urine samples from dogs receiving [^{3}H]equilin sulfate

Compound	Average percentage of radioactivity
17β-Dihydroequilin	31
17β-Dihydroequilenin	43
17α-Dihydroequilin	9
17α-Dihydroequilenin	7
Equilenin	4
Equilin	6

sulfate alone, or [^{3}H]equilin sulfate in a mixture of conjugated equine estrogens as in the Premarin® brand conjugated estrogen preparation, in this study. The results indicate that the drug is rapidly absorbed and it has a moderate half-life. Also, the results from this study indicate that the other components that are present in the conjugated estrogen preparation Premarin® alter the pharmacokinetics of equilin sulfate in dogs, since the kinetics of radioactivity were significantly different when it was administered as a single compound compared to when it was given in a mixture of conjugated equine estrogens.

A significant reduction in the urinary excretion of radioactivity when [^{3}H]equilin sulfate was administered in a mixture compared to when it was given alone suggests that the absorption and/or metabolism of equilin sulfate is influenced by the other components in conjugated equine estrogens. Following oral administration of doubly labeled equilin sulfate to humans, it has been shown that the sulfate of the majority of the dose is cleaved in the gut and the estrogen is absorbed in its phenolic (unconjugated) form. The estrogen is then reconjugated and circulates in blood mainly in the form of sulfate conjugate. Perhaps this hydrolysis and/or absorption step(s) is influenced by the other estrogens in the equine estrogens [6]. In fact, estriol has been shown to reduce the absorption of estrone and estradiol when they were co-administered orally to monkeys, leading to changes in pharmacokinetics [11]. Asch and Borghi hypothesized that estriol acts as an antagonist to estrone and estradiol at the enzymatic level of gastrointestinal mucosa.

Conjugated equine estrogens mixture contains at least ten estrogens. Following administration of this mixture to dogs, the hydrolyzed plasma and urine samples showed numerous peaks in the HPLC–electrochemical chromatograms. Because of the separation limitations of the HPLC method used in this

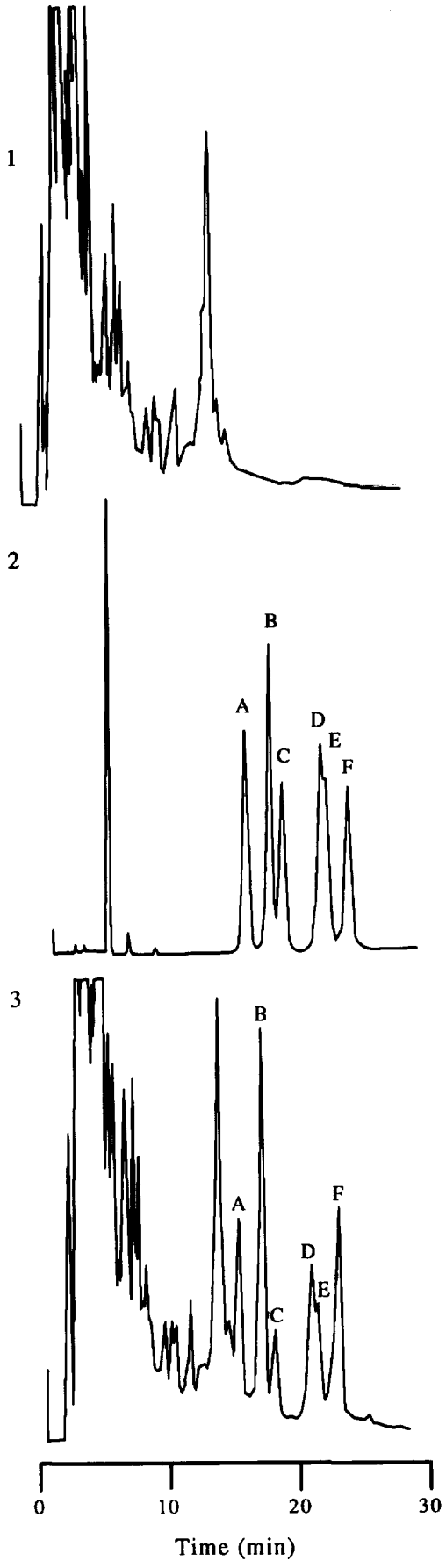

Fig. 4. HPLC–electrochemical chromatograms of: (1) hydrolyzed control plasma sample; (2) reference standards, 17β-dihydroequilenin (A), 17β-dihydroequilin (B), 17α-dihydroequilenin (C), 17α-dihydroequilin (D), equilenin (E), and equilin (F); and (3) 1 h plasma sample from a dog receiving 2.5 mg/kg of [^{3}H]equilin sulfate.

Fig. 4

Table 4. GC retention times and mass spectral data of equilin and its metabolites extracted from hydrolyzed 0–24 h urine from dogs receiving 2.5 mg/kg of [^{3}H]equilin sulfate

Compound	GC rt (min)	Mass spectral data of trimethyl silyl derivative*
Equilin	20.9	340 (100, M^+), 325 (10), 312 (10), 297 (10), 283 (20), 255 (20), 242 (20), 229 (20), 221 (22)
Equilenin	21.8	338 (100, M^+), 323 (15), 310 (20), 296 (45), 295 (40), 282 (40), 269 (30)
17β-Dihydroequilin	21.6	414 (100, M^+), 399 (10), 324 (20), 309 (40), 289 (30), 283 (25)
17β-Dihydroequilenin	22.5	412 (60, M^+), 397 (30), 369 (10), 322 (35), 307 (10), 296 (45), 281 (90), 267 (10), 230 (15), 213 (15)
17α-Dihydroequilin	21.3	414 (20, M^+), 399 (5), 324 (40), 309 (75), 289 (10), 281 (10), 267 (10), 229 (22)
17α-Dihydroequilenin	22.3	412 (90, M^+), 397 (5), 322 (35), 307 (100), 267 (5), 251 (5), 213 (5)

*Obtained by EI–GC–MS. Prominent fragments (m/z) with corresponding abundances (%) in parentheses.

study, only radioactivity could be measured following administration of [^{3}H]equilin sulfate in the conjugated estrogen mixture. However, metabolite profiles could still be determined by monitoring the radioactivity by a radioisotope detector.

Metabolite profiles indicate that the bulk of the metabolites was present as conjugates in both plasma and urine, and the conjugates were primarily in the glucuronide forms. Estrone and its metabolites are known to circulate as well as be eliminated mostly in the form of glucuronide conjugates in the dog [12]. As mentioned earlier, in humans, the estrogens circulate mostly as sulfate esters [6]. Structural characterization of phase I metabolites indicate that the reduction of the 17-ketone group to form both 17β- and 17α-dihydro products (diols) and aromatization of ring-B are the major metabolic pathways for equilin sulfate in dogs (Fig.5). The metabolism of equilin in the dog seems to be similar to that in the mare [7]. In contrast, only 17β-reduced metabolites are formed in humans [3, 6]. However, 17α-dihydro estrogens are present in Premarin®.

The 17β-reduced estrogens are in general known to be more potent than the 17-keto estrogens in the uterotropic estrogenicity assays [3, 7]. On the other hand, the 17α-reduced unsaturated estrogens are now known to be potent cardiovascular agents in animal models. For example, 17α-dihydroequilin sulfate is efficacious against atherosclerosis in cholesterol fed rabbits and 17α-dihydroequilenin lowers the total plasma cholesterol by greater than 60% in rats [13, 14]. Therefore, each of the metabolites of equilin sulfate that have been identified in the dog may play a role in the biological activities of this estrogen. There is some similarity between the biotransformation of equilin and estrone, an endogenous estrogen in the dog. Both estrogens lead to the formation of 17α- and 17β-reduced products. However, equilin is not metabolized to its 3,16,17-trihydroxy estrogens as estrone is known

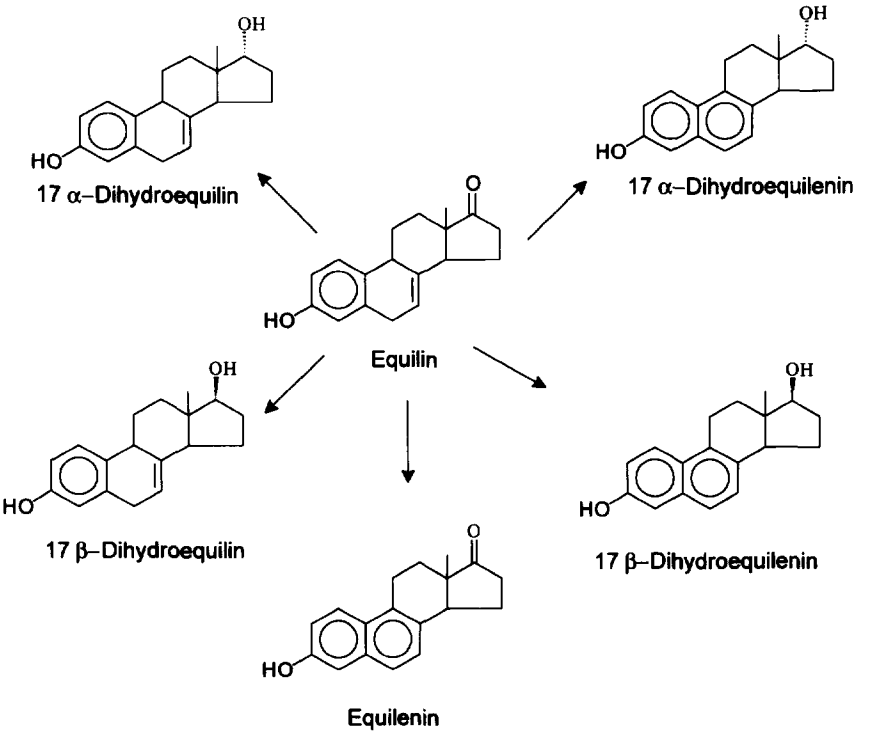

Fig. 5. Metabolic pathways of equilin in dogs.

to be metabolized to estriol and 17 epi-estriol in the dog [15].

In conclusion, following oral administration of [^{3}H]equilin sulfate to dogs, the radioactivity is rapidly absorbed. The disposition of radioactivity is altered by the other components of the equine estrogen preparation Premarin®. The reduction of the 17-keto group and aromatization of ring-B are the major metabolic pathways of equilin in the dog.

Acknowledgements—The authors wish to acknowledge the technical assistance from Ms J Sheehan and the mass spectrometry instrumental support from Mr J. Lech and Dr R. T. Rosen of Center for Advanced Food Technology, Mass Spectrometry Lab, Rutgers University, New Brunswick, New Jersey, U.S.A. The Center for Advanced Food Technology is a member of the New Jersey Commission for Science and Technology.

REFERENCES

1. Ansbacher R.: Bioequivalence of conjugated estrogen products. *Clin. Pharmacokinet.* **24** (1993) 271–274.
2. Lyman G. W. and Johnson R. N.: Assay for conjugated estrogens in tablets using fused silica capillary gas chromatography. *J. Chromatogr.* **234** (1982) 234–239.
3. Bhavnani B. R.: The saga of the ring-B unsaturated equine estrogens. *Endocrine Rev.* **9** (1988) 396–416.
4. Bhavnani B. R. and Woolever C. A.: Interaction of ring-B unsaturated estrogens with estrogen receptors of human endometrium and rat uterus. *Steroids* **56** (1991) 201–210.
5. Lobo R. A., Nguyen H. N., Eggena P. and Brenner P. F.: Biologic effects of equilin sulfate in post-menopausal women. *Fertil. Steril.* **49** (1988) 234–238.
6. Bhavnani B. R., Woolever C. A., Wallace D. and Pan C. C.: Metabolism of [^{3}H]equilin [^{35}S]sulfate and [^{3}H]equilin sulfate after oral and intravenous administration in normal postmenopausal women and men. *J. Clin. Endocr. Metab.* **68** (1989) 757–765.
7. Bhavnani B. R. and Woolever C. A.: *In vivo* metabolism of [^{3}H]equilin in the pregnant mare. *Endocrinology* **108** (1981) 232–238.
8. Rocci M. and Jusko W. J.: LAGRAN program for area and moments in pharmacokinetic analysis. *Comput. Prog. Bio-Med.* **16** (1983) 203–216.
9. Gibaldi M. and Perrier D.: Non-compartmental analysis based on statistical moment theory. In *Pharmacokinetics*, 2nd Edition. Marcel Dekker, Inc. (1982) pp. 409–417.
10. Roman R., Yates C. H., Millay J. F. and Vandenheuvel N. J. A.: Identification of estrogens isolated from pregnant mare's urine. *Can. J. Pharmac. Sci.* **10** (1975) 8–11.
11. Asch R. H. and Borghi M. R.: Estriol modifies the absorption of estrone and estradiol in castrated rhesus monkeys: a new therapeutic approach for the use of natural estrogens in the menopause? *J. Steroid Biochem.* **20** (1984) 995–998.
12. Collins D. C.: Identification of conjugated estrogen metabolites in dog plasma following administration of estrone-6,7-H. *Steroids* **18** (1971) 341–358.
13. Sulistiyani, Adelman S. J., Chandrasekaran A., Jayo J. and St Clair R. W.: Effect of 17α-dihydroequilin sulfate, a conjugated equine estrogen and ethinyl estradiol, on atherosclerosis in cholesterol fed rabbits. *Arteriosclerosis Thromb. Vasc Biol.* **15** (1995) 837–846.
14. Washburn S. A., Adams M. R., Clarkson T. B. and Adelman S. J.: A conjugated equine estrogen with differential effects on uterine weight and plasma cholesterol in the rat. *Am. J. Obstet. Gynec.* **969** (1993) 251–259.
15. Collins D. C., Balikian H. M. and Preedy R. K.: Dynamics of estrone glucosiduronate metabolism in dogs. *Steroids* **26** (1975) 757–768.