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Short Communication

Identification of four plasma metabolites of Etoperidone in dogs

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Introduction

Etoperidone hydrochloride (Et), 2-(3-(4-(3-chlorophenyl)-1-piperazinyl)propyl)-4,5-diethyl-2,4-dihydro-3H-1,2,4-triazol-3-one monohydrochloride, is an antidepressant agent marketed in Europe. Et modulates serotonergic function *in vivo* and *in vitro* [1–6]. Et is well absorbed, and extensively metabolized in animals and humans after oral administration [6–8]. The structure of Et is closely related to that of trazodone. Both Et and trazodone form a major metabolite, 1-(3-chlorophenyl)piperazine, (MCPP), which may contribute to the antidepressent effects of the parent drugs [3, 4, 9–12]. The present work describes the isolation, characterization and identification of unchanged Et and four plasma metabolites in dogs utilizing chromatography (column, HPLC, TLC) and spectroscopy (MS, NMR) by comparison to synthetic references. A preliminary report of this work was recently presented [13].

Experimental

A single solution dose of Et hydrochloride (20 mg kg^{-1}, free base) was administered orally to four male beagle dogs (10–15 kg), and plasma (50 ml) was collected at 1 h post-dose. Et and its metabolites were extracted from alkalinized plasma as described in Fig. 1. The extract residue was reconstituted in hexane–dichloromethane (1:1) and 10% of this sample solution was evaporated to dryness, reconstituted in methanol, and used for HPLC metabolite profiling (Fig. 2). The remaining plasma extract solution was chromatographed over a neutral alumina column (Figs 1, 3). The residues from the collected fractions were further separated by TLC using the conditions indicated in Fig. 3. TLC analysis of samples was conducted on silica gel GF plates (5 × 20 cm; 250 μm; Analtech, Newark, DE, USA). LC systems used were a Beckman Model 114 pump, Water Assoc. — WISP autoinjector, and Kratos Spectroflow 783 UV detector (254 nm). Separation of Et and metabolites was accomplished using the conditions described in Fig. 2. The percentage of Et or each metabolite was estimated using the percentage peak height/total peak heights from its UV absorbance. Electron-impact (EI) mass spectra were obtained in a VG7070E (VG Micromass, Manchester, UK). Chemical-ionization (CI) mass spectra were obtained on a Finnigan Model 3300 (Finnigan-MAT Inc., San Jose, CA, USA). Operating conditions for

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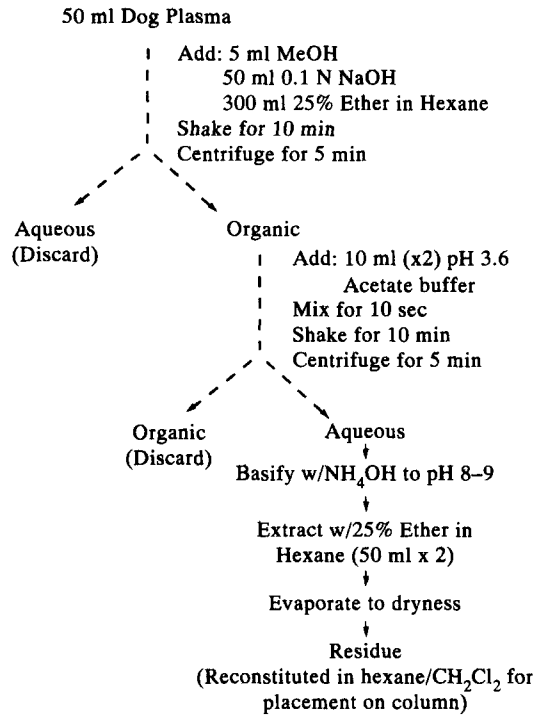


Figure 1
Plasma extraction of Etoperidone.

spectrometers included: source temperature — 200°C; reagent gas (ammonia) pressure for CI — 2 torr; ionization potential — 70 eV; multiplier voltage — 1.8 KeV; electrometer setting — 10 A/V; sample inlet — desorption probe; sample size — 5 μl. NMR spectra were determined in deuterochloroform in a Bruker Model WM 360 (Bruker Instrument, Inc., Billerica, MA, USA). Et hydrochloride, 5-(1-hydroxyethyl)etoperidone (RWJ-36766), 5-(1-oxoethyl)etoperidone (RWJ-36771) and MCPP (RWJ-24315) were synthesized at the R.W. Johnson Pharmaceutical Research Institute (Spring House, PA, USA).

Results and Discussion

The LC methodology described above afforded good recovery and separation of unchanged Et and its metabolites along with the seeded internal standard, trazadone, in plasma (Fig. 2) [8]. The plasma concentration of unchanged Et ranged from 2 to 1000 ng ml^{-1} in dogs postdose [8]. Unchanged Et and four plasma metabolites (two major, two minor) were isolated from the plasma pool using LC, column chromatography and TLC. The isolated Et and metabolites were identified on the basis of MS (CI, EI) and ^1H NMR data by comparison with synthetic samples. Unchanged Et was isolated as a major plasma component (24% of nonphenolic sample fraction) using column chromatography or an LC peak collecting method. Its LC, TLC, MS and NMR data were identical to those of authentic Et. The MS analysis of unchanged Et provided an intense protonated molecular ion at *m/z* 378 (100% — relative intensity) in CI and a weak molecular ion at *m/z* 377 (5%) in EI, along with informative fragment ions at

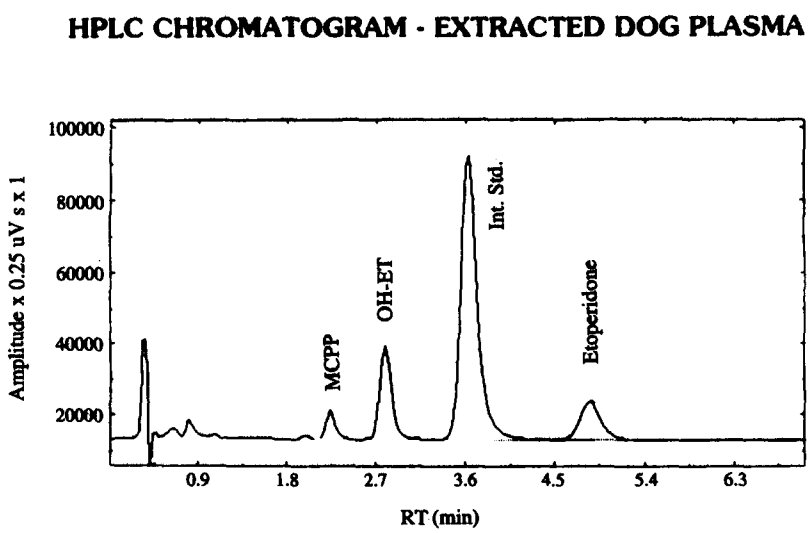


Figure 2
LC metabolic profilie of plasma. Column: Analytichem® Sepralyte C18 (3 μm, 5.0 cm × 4.5 mm i.d. with Brownlee C18 guard column (7 μm, 33.0 cm × 4.6 mm i.d.). Detector: λ = 254. Mobile phase: 30% methanol; 10% acetonitrile; 60% acetate buffer (0.14 M, pH 3.6) with N-pentylamine (0.1 M) and heptanesulphonic acid, sodium salt (0.005 M). Flow rate: 2 ml min^{-1}.

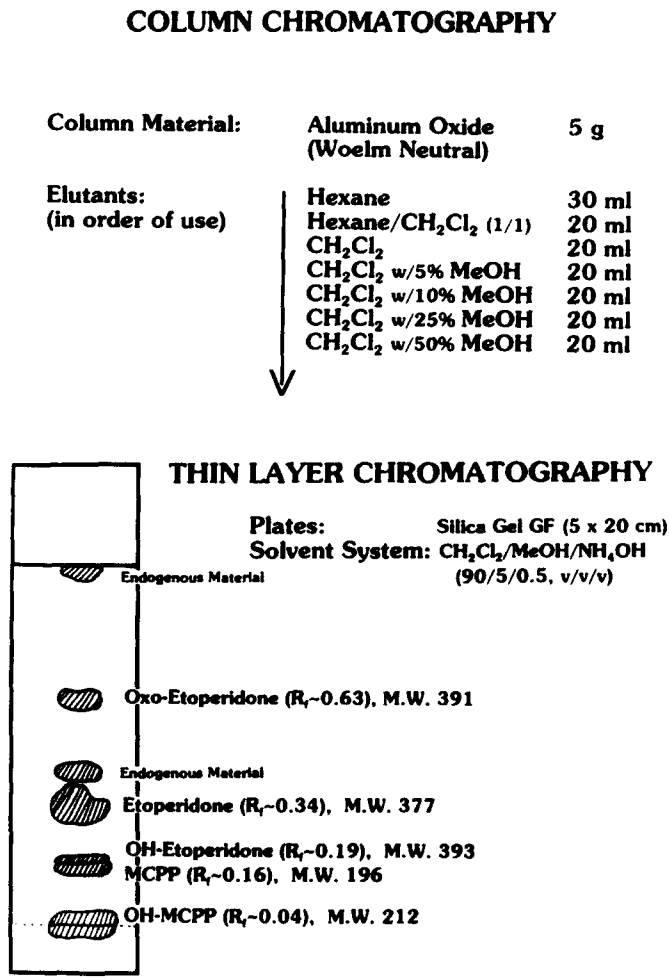
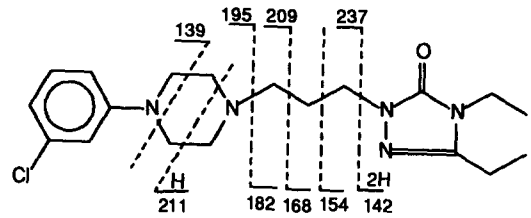


Figure 3
Column chromatography conditions and TLC profile for plasma metabolites of Etoperidone.

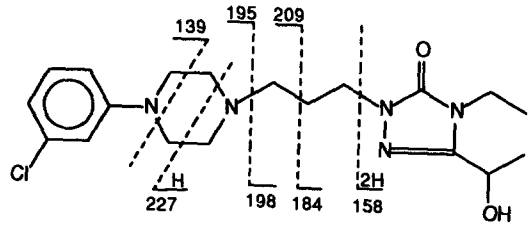
m/z 362 ($M^{+\cdot}$–Me; 5% EI), 237 (2% CI, 14% EI), 225 (1% CI, 10% EI), 211 (12% CI, 100% EI), 195 (2% CI, 5% EI), 182 (5% CI, 27% EI), 168 (3% CI, 15% EI), 154 (2% CI, 9% EI), 142 (3% CI, 11% EI) and 139 (3% CI, 10% EI) (Figs 4, 5). The ^{1}H NMR data of Et are shown in Fig. 6.

5-(1-Hydroxyethyl)etoperidone, a major plasma metabolite (50% of nonphenolic sample fraction), was isolated by LC, column and TLC, and analysed by CI-MS, EI-MS and NMR. CI(ammonia)-MS and EI-MS of the hydroxylated metabolites gave an intense protonated molecular ion and a molecular ion at *m/z* 394 (base peak) and *m/z* 393 (5%), respectively (Fig. 4). Important fragment ions at *m/z* 257 (4% CI), 253 (7% CI, 12% EI), 227 (55% CI, base peak EI), 209 (25% CI, 29% EI), 198 (20% CI, 25% EI), 195 (6% CI, 5% EI), 184 (4% CI, 8% EI), 158 (18% CI, 8% EI) and 139 (19% CI, 9% EI) were consistent with the addition of a hydroxy group to the triazole ring of Et (Figs 4, 5). The proton NMR spectrum (Fig. 6) revealed a quartet resonance with an integration of one proton at δ 4.70. This indicated a secondary alcohol located at the 1-carbon of the 5-ethyl group. Direct comparison of the TLC, LC, NMR and MS data of the isolated metabolite with a synthetic sample (RWJ-36766) demonstrated that they were identical.

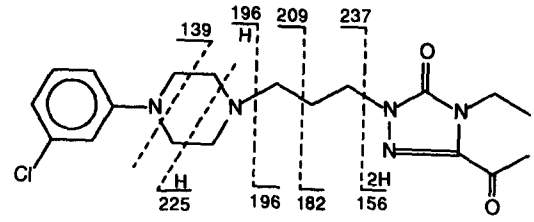
5-(1-Oxoethyl)etoperidone, a minor plasma metabolite (<2% of nonphenolic sample fraction) produced an intense protonated molecular ion at *m/z* 392 (MH^+, base peak) and an apparent molecular ion at *m/z* 391 (20%) in CI (ammonia) and EI-MS, respectively, which is a 14 amu increase from Et (Figs 4, 5). Informative fragment ions revealed at *m/z* 251 (7% CI, 15% EI), 237 (11% EI), 225 (62% CI, base peak EI), 209 (35% CI), 196 (22% CI, 25% EI) and 139 (21% CI, 33% EI)



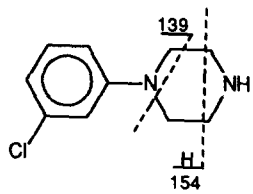
Etoperidone



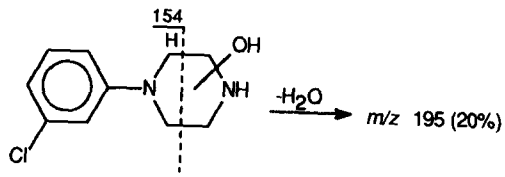
5-(1-Hydroxyethyl)etoperidone



5-(1-Oxo-ethyl)etoperidone



MCPP



OH-MCPP

Figure 4
Structure of etoperidone, its metabolites and their MS fragment ions.

are suggestive of an oxo-group located at the 1-carbon of the 5-ethyl group (Fig. 4). The NMR spectrum (Fig. 6) of the isolated metabolite indicated a singlet resonance for an acetyl group at δ 2.45. The structure of this metabolite was confirmed on the basis of its TLC, MS and ^{1}H NMR characteristics by comparison to those of a synthetic sample (RWJ-36771).

Meta-chlorophenylpiperazine (MCPP), a major plasma metabolite (15% of nonphenolic sample fraction), was isolated by LC, column and TLC, and identified based on TLC, LC, MS and NMR data by comparison with an authentic sample (RWJ-23415). The CI(ammonia)-MS exhibited an intense ammonium-adduct molecular ion and a protonated molecular ion at *m/z* 214 (base

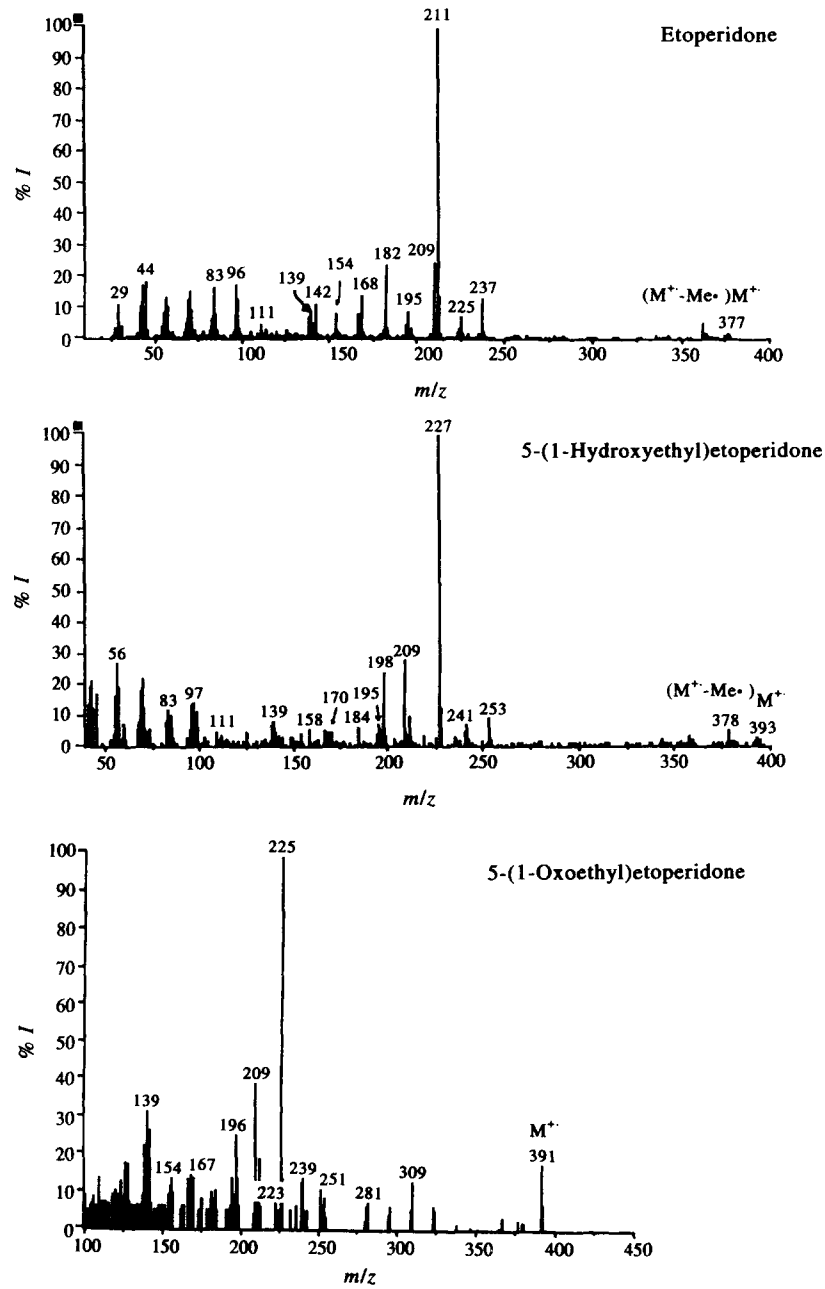


Figure 5
EI mass spectra of Etoperidone, 5-(1-hydroxyethyl)etoperidone, and 5-(1-oxo-ethyl)-etoperidone.

peak) and 197 (3%), respectively, along with fragment ions at *m/z* 155 (12%) and 112 (4%) (Fig. 4).

Hydroxy-MCPP was isolated as a minor metabolite (<2% of nonphenolic sample fraction) and tentatively elucidated by CI-MS data only. The CI(ammonia) data indicated an intense protonated molecular ion at *m/z* 213 (base peak) together with prominent fragment ions at *m/z* 195 (MH^+-H_2O, 20%) and 154 (8%) (Fig. 4) which led to the assignment of the structure of this isolated metabolite as an hydroxylated MCPP. The exact position of the hydroxyl group was uncertain, but was tentatively assigned to the piperazinyl ring. The alkaline aqueous fraction probably contained the phenolic, acidic, N-oxide, or conjugated metabolites which were not investigated in this study.

Conclusions

The proposed metabolic pathways for the circulating plasma metabolites of Et in the dog

Compound	Phenyl proton	Chemical Shift (δ)								
		$-CH_2-N<$ $N=$	$>N-CH_2CH_3$	$ph-N<$ CH_2- CH_2-	$-CH_2-$ $-CH_2-N<$	$N-CH_2-$	$C-CH-CH_3$ (R)	$-CH_2-$	N CH_2CH_3	C RCH_3
							R = H			R = CH_2
Etoperidone	6.75-7.40 (m, 4H)	3.80 (t, 2H)	3.65 (q, 2H)	3.22 (t, 4H)	2.63 (t, 4H)	2.60 (t, 2H)	2.52 (q, 2H)	2.00 (q, 2H)	1.25 (t, 3H)	1.23 (t, 3H)
							R = OH			R = CHOH
OH-Et	6.74-7.25 (m, 4H)	3.81 (t, 2H)	3.50 (q, 2H)	3.20 (t, 4H)	2.55 (t, 4H)	2.42 (t, 2H)	4.72 (q, 2H)	1.90 (q, 2H)	1.25 (t, 3H)	1.55 (d, 3H)
										R = C(=O)
Oxo-Et	6.75-7.15 (m, 4H)	4.02 (t, 2H)	3.85 (q, 2H)	3.17 (t, 4H)	2.52 (t, 4H)	2.45 (t, 2H)	–	1.95 (q, 2H)	1.22 (t, 3H)	2.45 (s, 3H)
MCPP	6.75-7.20 (m, 4H)	–	–	3.20 (t, 4H)	2.00 (t, 4H)	–	–	–	–	–

s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet

Figure 6
^{1}H NMR spectral data for Etoperidone and its metabolites.

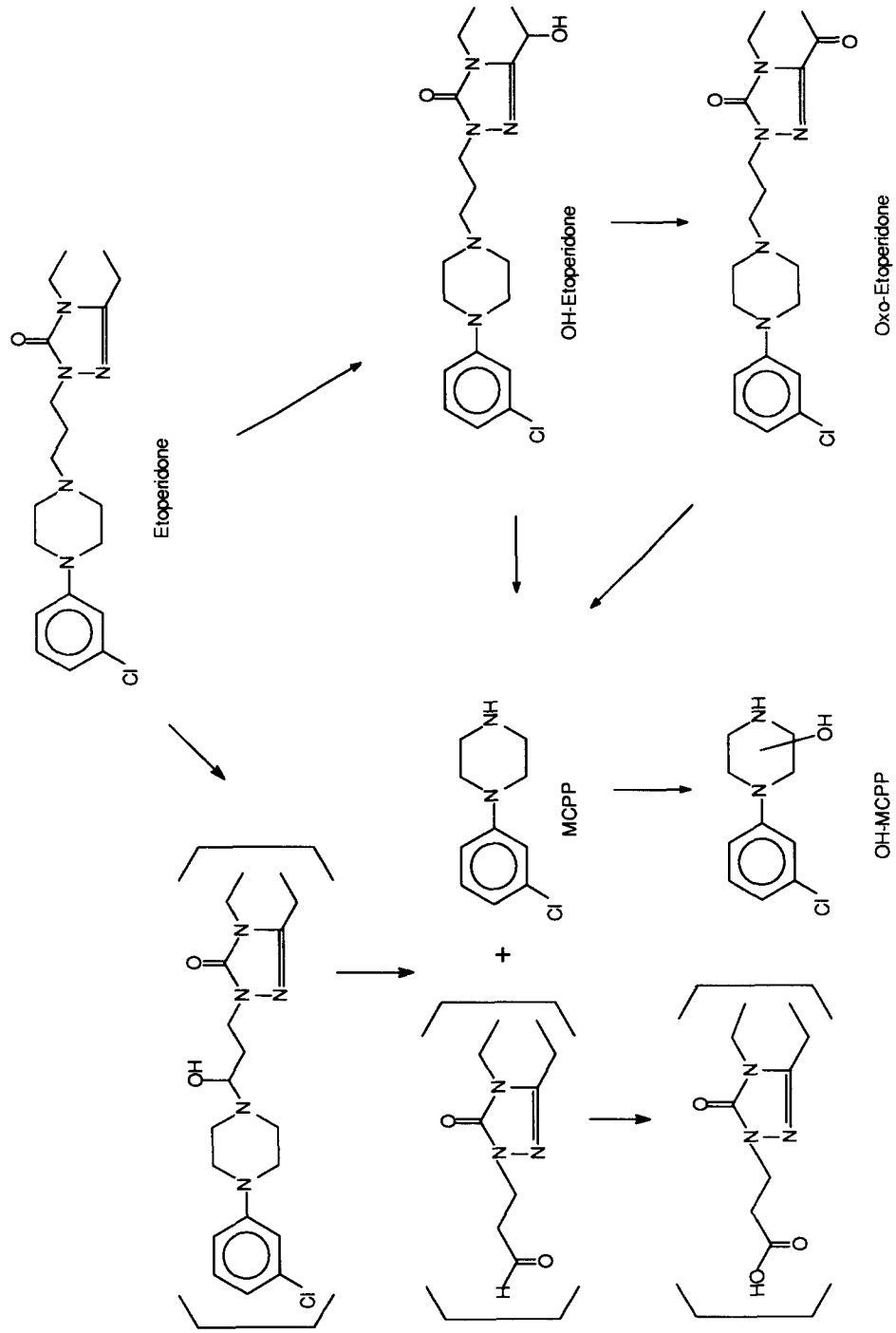


Figure 7
Proposed metabolic pathways for circulating plasma metabolites of Etoperidone in the dog.

are shown in Fig. 7. 5-(1-Hydroxyethyl)etoperidone was formed as a major metabolite (50% of nonphenolic sample fraction) from the oxidation of Et at the 1-carbon of the 5-ethyl group. Further oxidation of this hydroxylated metabolite produced 5-(1-oxoethyl)etoperidone as a minor plasma metabolite (<2% of nonphenolic sample fraction). Oxidative N-dealkylation of Et, 5-(1-hydroxyethyl)etoperidone, or 5-(1-oxoethyl)etoperidone led to cleavage and the formation of the biologically active metabolite, MCPP, in major quantities (15% of nonphenolic sample fraction). Oxidation of MCPP at the piperazine ring produced hydroxy MCPP in minor quantities (<2% of nonphenolic sample fraction).

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References

[1] M.T. Ramacci, O. Ghirardi, F. Maccari, L. Pacifici and P. Sale, *Arzneim-Forsch.* **29**, 294–307 (1979).
[2] E. Przegalinski and A. Lewandowska, *J. Neural Transm.* **46**, 303–312 (1979).
[3] V. Cioli, C. Carradino, D. Piccinelli, W.G. Rocchi and P. Valeri, *Pharmacol. Res. Commun.* **16**, 85–100 (1984).
[4] G.E. Martin, J.R. Mathiasen and J.M. Kesslick, *Psychopharmacology* **99**, 94–97 (1989).
[5] R.B. Raffa, R.P. Shank and J.L. Vaught, *Psychopharmacology* **108**, 320–326 (1992).
[6] C. Bolden-Watson and E. Richelson, *Life Sci.* **52**, 1023–1029 (1993).
[7] W.N. Wu, G.W. Caldwell and J.A. Uetz, *Pharmacologist* **33**, 208 (1991).
[8] M.L. Holland and E.T. Heebner, *J. Chromat.* **567**, 433–440 (1991).
[9] S. Caccia, *Boll. Chim. Farm.* **129**, 183–189 (1990).
[10] S. Garattini, *Clin. Pharmacokinet.* **10**, 216–217 (1985).
[11] M.H. Fong, S. Garattini and S. Caccia, *J. Pharm. Pharmacol.* **34**, 674–675 (1982).
[12 M. Melzacka, A. Rurak and J. Vetulani, *Pol. J. Pharmacol. Pharm.* **32**, 551–556 (1980).
[13] W.N. Wu, J.A. Masucci, J.D. Rodgers and L.A. McKown, *The 4th International Symposium on Pharmaceutical and Biomedical Analysis Abstract 19* (1993).