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In vivo biotransformation of fenoctimine in rat, dog and man

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1. The metabolism of fenoctimine (Fn) was studied in rat, dog and man following administration of ^{14}C-Fn sulphate.

2. Seventeen Fn metabolites were isolated by hplc and tlc from rat bile, dog bile, dog urine, human urine, human faecal extracts, and human plasma and identified using nmr and MS.

3. The identified metabolites accounted for 75% of total radioactivity in rat bile, 80% in dog bile, and 40% in dog urine samples. In man, 90% of the urinary, 70% of the faecal, and > 50% of the plasma total radioactivity were identified.

4. Three major pathways for Fn metabolism were proposed. These pathways involved imino-bond cleavage, aromatic hydroxylation and oxidation of the aliphatic chain.

5. The imino-bond cleavage pathway was dominant in all species. However, the other two pathways differed in quantitative importance among the species studied.

6. The aromatic hydroxylation pathway appeared to be the most important means of biotransformation of Fn in dog since all but two of the metabolites were formed by this route.

7. The aliphatic oxidation pathway appeared to be important to the biotransformation of Fn in man and produced three major metabolites.

Introduction

Fenoctimine (Fn) sulphate is a novel compound possessing gastric antisecretory activity in rat, dog, pig, guinea pig and man (Jacoby *et al.* 1982, Williams *et al.* 1981, 1983). Preliminary results on the disposition of Fn in mouse, rat, rabbit, dog and man have been reported (Wu *et al.* 1985, Yorgey *et al.* 1986, Renzi *et al.* 1983, McKown *et al.* 1992). The extent of metabolism of Fn was > 95% in all species studied.

This paper describes the isolation and identification of Fn and 17 metabolites in plasma, urine, bile, and faeces from the rat, dog and man

Materials and methods

Chemicals and materials

^{14}C-Fn · H_2SO_4,4-[diphenyl(^{14}C-)methyl]-1-[(octylimino)methyl]piperidine sulphate was synthesized at the The R. W. Johnson Pharmaceutical Research Institute (Spring House, PA, USA) with a radiochemical purity > 97% (by radio tlc). The drug was used at a specific activity of 6·5 μCi/mg for the rat and dog and 0·516 μCi/mg for man.

Fn · H_2SO_4, 4-hydroxy-Fn (**XII**), 7-oxo-Fn (**XIV**), 8-hydroxy-Fn (**XV**), 8-carboxy-Fn (**XVI**), 6-hydroxy-Fn (**XVII**), 4-(diphenylmethyl) piperidine (**IV**), 4-hydroxy-4-(diphenylmethyl) piperidine (**V**) and 1-formyl-4-(diphenylmethyl)-piperine (**I**) were also synthesized at the R. W. Johnson Pharmaceutical Research Institute (each with chemical purity > 95% by hplc) (Scott *et al.* 1987).

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Diazald, used to generate diazomethane, was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Glusulase, a mixture of arylsulphatase and β-glucuronidase (1:4, v/v) from *Helix pomatia*, was obtained from Endo Laboratories, Inc. (Wilmington, DE, USA).

Type II β-glucuronidase from *Escherichia coli* and type H-2 β-glucuronidase from *H. pomatia* were purchased from Sigma Chemical Co. (St Louis, MO, USA). Saccharo-1,4-lactone (a glucuronidase inhibitor) was obtained from Calbiochem-Behring Corp. (La Jolla, CA, USA). Biofluor was purchased from New England Nuclear (Boston, MA, USA), and phosphomolybdic acid was obtained from Fisher Scientific Co. (Fairlawn, NJ, USA). BSTFA [*N,O*-bis-(trimethylsilyl)-trifluoroacetamide] with 1% TMCS (trimethylchlorosilane) was purchased from Pierce Chemical Co. (Rockford, IL, USA). Amberlite XAD-2 was purchased from Rohm and Haas (Philadelphia, PA, USA). Sephadex LH-20 (25–100 μm particle size) was obtained from Pharmacia Fine Chemicals (Piscataway, NJ, USA). Hplc grade solvents were obtained from Fisher and glass distilled solvents were purchased from Burdick and Jackson Laboratories, Inc., (Muskegon, MI, USA). Other reagent grade chemicals were obtained from commercial sources and were used without further purification.

Analytical equipment

Thin-layer radiochemical analyses were performed used 5 × 20 cm silica gel GF thin-layer plates (250 μm gel thickness, Analtech, Inc., Newark, DE, USA). Tlc plates were analysed using a Berthold Model LB276 radioscanner (Wildbad, Germany). Total radioactivity in each sample before and after purification was determined by liquid scintillation counting (LSC) of an appropriate aliquot of each sample in 10 ml Biofluor. The samples were counted in a refrigerated Searle Analytic 81 instrument (Downers Grove, IL, USA).

Liquid chromatographic (hplc) analyses were performed using an Altex Scientific System (Berkeley, CA, USA) equipped with a model 110 solvent metering pump, a model 153 UV detector (254 nm), a Water Associates (Milford, MA, USA) U6K injector and a model Flow I radioactive flow detector (Radiomatic Instruments, Tampa, FL, USA). A Hibar 10 μm LiChrosorb RP-18 column (25 cm × 4·6 mm id) (E. Merck, Darmstadt, Germany) and a MPLC 5 μm Spherisorb Silica Cartridge (13 cm × 4·6 mm id) (Brownlee Lalis, Inc., Santa Clara, CA, USA) were used.

Electron impact (EI) mass spectrometric analyses were performed using a Vg 7070 mass spectrometer (Vg Micro Mass, Manchester, UK). Chemical ionization (CI) mass spectrometric analyses were performed using methane or ammonia as the reagent gas in the Vg 7070 or the Finnigan 3300/6100 mass spectrometer (Sunnyvale, CA, USA).

Proton nuclear magnetic resonance spectra (NMR) were obtained using a JEOL Fourier Transform Spectrometer (Model FX60Q, JEOL, Analytical Instrument Division, Cranford, NJ, USA) or a Bruker Fourier Transform Spectrometer (Model WM 360, Bruker Instrument, Inc., Manning Park, Billerica, MA, USA).

Sample source

A total of six sample pools were used in this study; bile pools from rat, bile and urine pools from dog and plasma, urine and faecal extract pools from man. The sampling interval and the percent of the administered dose recovered in each pool are presented in table 1.

For rat, the bile pool was a mixture of bile collected over 24 h from two male rats that received ^{14}C-fenoctimine sulphate and 22 male rats that were given non-radiolabelled fenoctimine sulphate intraperitoneally (ip) as single 15-mg/kg suspension doses. Bile was obtained quantitatively by cannulating the bile duct of each rat.

Dog bile and urine pools were obtained by mixing the respective fluids from one anaesthetized dog that received a 50-mg/kg dose intravenous (iv) infusion dose of ^{14}C-fenoctimine sulphate and five anaesthetized dogs that each received single 50-mg/kg dose iv infusion doses of unlabelled fenoctimine sulphate. Bile samples were quantitatively collected from bile duct cannulae 0–7 h after dosing and urine was collected at the end of the 7 h collection period by aspiration of the bladder of each dog.

Human plasma, urine, and faecal extract pools were composed of equal aliquots or fractions of samples from each of six subjects who received a single oral 150-mg/subject solution dose of ^{14}C-fenoctimine sulphate solution during the human excretion study (Renzi *et al.* 1983). The doses given to animals were the mid-dose of toxicity studies in animals investigated.

Sample preparation

All samples were purified prior to tlc and hplc analysis, using the procedures shown in table 1. At least one of the following two treatments was used on portions of each sample: (1) treatment I involved glusulase treatment of an aqueous sample for 18 h at 38°C after the sample pH had been adjusted to pH 5·1 with glacial acetic acid and mixed with 1 M sodium acetate buffer (pH 5·1). The glusulase-treated sample was then extracted with ethyl acetate in an attempt to obtain any aglycones released from the sample after enzyme hydrolysis, and (2) treatment II involved sample evaporation or lyophilization. The residue was then dissolved in methanol and treated with ethereal diazomethane at room temperature. This treatment was designed to methylate phenolic hydroxy or carboxy groups.

In some cases the untreated or glusulase-treated samples were applied without purification to 250 μm

Table 1. Sources of pooled samples, purification procedures and subsequent treatments used for tlc and hplc analysis.

Species	Dose	Sample (% of administered dose)		Sampling interval (h)	Purification procedure (% of sample recovered)		Treatment
Rat	15 mg/kg in 0·5% methyl cellulose (ip)	bile	(22·9)	(0–24)	A	(91)	none, I, II
Dog	50 mg in 5% dextrose (iv)	bile	(18·5)	(0–7)	A	(74)	none, I, II
		urine	(3·4)	(0–7)	A	(90)	I,
Man	150 mg in water (po)	urine	(24·6)	(0–24)	B then A	(95)	I, II
		faecal extracts	(43·1)	(24–72)	C then A	(61)	I, II
		plasma		(1–48)	D	(75)	I, II

Purification procedures: A, extract aqueous sample with XAD-2 resin, extract resin with methanol, evaporate to dryness, reconstitute with water, purify on Sephadex LH-20 column, elute fractions with water; B, ether wash; C, evaporate sample to dryness, extract with water, wash extract twice with ether; and D, lyophilize sample, extract with methanol, evaporate to dryness, reconstitute with water, purify with Sephadex LH-20 column.

Treatments: I, adjust aqueous sample to pH 5·1, treat with Glusulase®, extract with ethyl acetate; and II, evaporate sample, place in methanol, treat with diazomethane.

thick tlc plates and chromatographed. After thorough drying, selected sections of silica gel were removed from the plates and the absorbed compounds eluted with a mixture of ethyl acetate and methanol. These extracts were then analysed using hplc.

Metabolite isolation and identification

Each hplc analysis was performed at 254 nm using an isocratic solvent system consisting of methanol and concentrated ammonium hydroxide (either 99·5/0·5 or 99/1, v/v). The flow rate was maintained at 2·0 ml/min for profiling, while flows of 1·0 and 0·5 ml/min were used during fraction collection.

Fractions containing the largest quantities of radioactivity in each metabolite peak were reinjected onto the column to monitor the purity (at 254 nm). The fraction containing a single component was evaporated using nitrogen gas to dryness. The residues were further analysed by MS or nmr.

The thin-layer radiochromatographic (tlc) analyses of the samples were carried out using rectangular glass chromatography tanks and silica gel GF thin-layer plates (250 μm). Two solvent systems were used for analysis. System A was a mixture of ethyl acetate/isopropanol/58% ammonium hydroxide (80:20:2, v/v/v) and system B was a combination of chloroform/methanol/water (35:60:5, v/v/v). Each tlc plate was developed to a height 14 cm above the sample application. Reference compounds were chromatographed parallel to the samples on the tlc plate. All plates were air dried following development and the samples and reference compounds were visualized using short wave-length UV light. Each tlc plate was analysed using the radioscanner. The radioactive bands from each tlc plate were individually removed by zone scraping and extracted with a mixture of ethyl acetate and methanol (80/20, v/v). The isolated products from the extracts were analysed by hplc, MS or nmr.

Metabolites were characterized by direct inlet CI (CH_4 or NH_3) and EI-MS. Nmr spectra of metabolites were obtained in deuterated chloroform or deuterated dimethylsulphoxide and chemical shifts were reported in ppm (δ) downfield from the tetramethylsilane (TMS) internal standard.

The percent recovery of each metabolite in a sample was determined from tlc or hplc analyses dependent upon the resolution obtained by each method. Each metabolite was identified by a combination of two or more characteristics determined from tlc, hplc, EI- and CI-MS, and nmr analyses.

Results

Fenoctimine and 17 metabolites were isolated from rat, dog and human samples. The structures and the chemical name of these compounds are shown in figure 1 along with the MS fragments; their tlc R_f's and the hplc retention times (R_t) are tabulated in tables 2 and 3 respectively. The sources of each metabolite are shown in table 4 along with the percent of the total radioactivity represented by each. The identified metabolites represent substantial portions of the total radioactivity in each sample analysed. The total percentages of doses and radioactivity in samples accounted for by the metabolites are summarised in table 4.

Trace amounts of fenoctimine were found in human plasma and faecal extracts. In rat bile, fenoctimine was detected after glusulase hydrolysis, indicating it was present as conjugated forms in minor amounts. It probably forms as fenoctimine-*N*-glucuronide. The identity of fenoctimine was confirmed by tlc and MS analyses by comparison with authentic fenoctimine. EI-MS analysis of fenoctimine exhibited a molecular ion at m/z 390, which was further supported by an intense protonated molecular ion at m/z 391 (100%) in CI. Prominent ions at m/z 250, 167 and 140 (100%) were consistent with the fragmentation patterns from fenoctimine (figure 1).

Metabolite **I** was isolated from dog bile and urine, and from human plasma, urine, and faecal extracts. Identification was obtained by CI and EI-MS (figure 1), which revealed an intense protonated molecular ion at m/z 280 (MH^+, 100%) and a molecular ion at 279 ($M^{+\cdot}$). Three additional fragments at m/z 202 (EI and CI), 167 (EI (100%) and CI), and 112 (EI) were prominent. The hplc and tlc characteristics and the spectra of this metabolite agreed with those obtained from an authentic sample.

Metabolite **II** was isolated from rat bile, dog bile and urine, and human plasma, urine and faecel extracts. A positive test for a phenol was observed on tlc using phosphomolybdic acid. The CI-MS of the isolated material (figure 1) indicated an

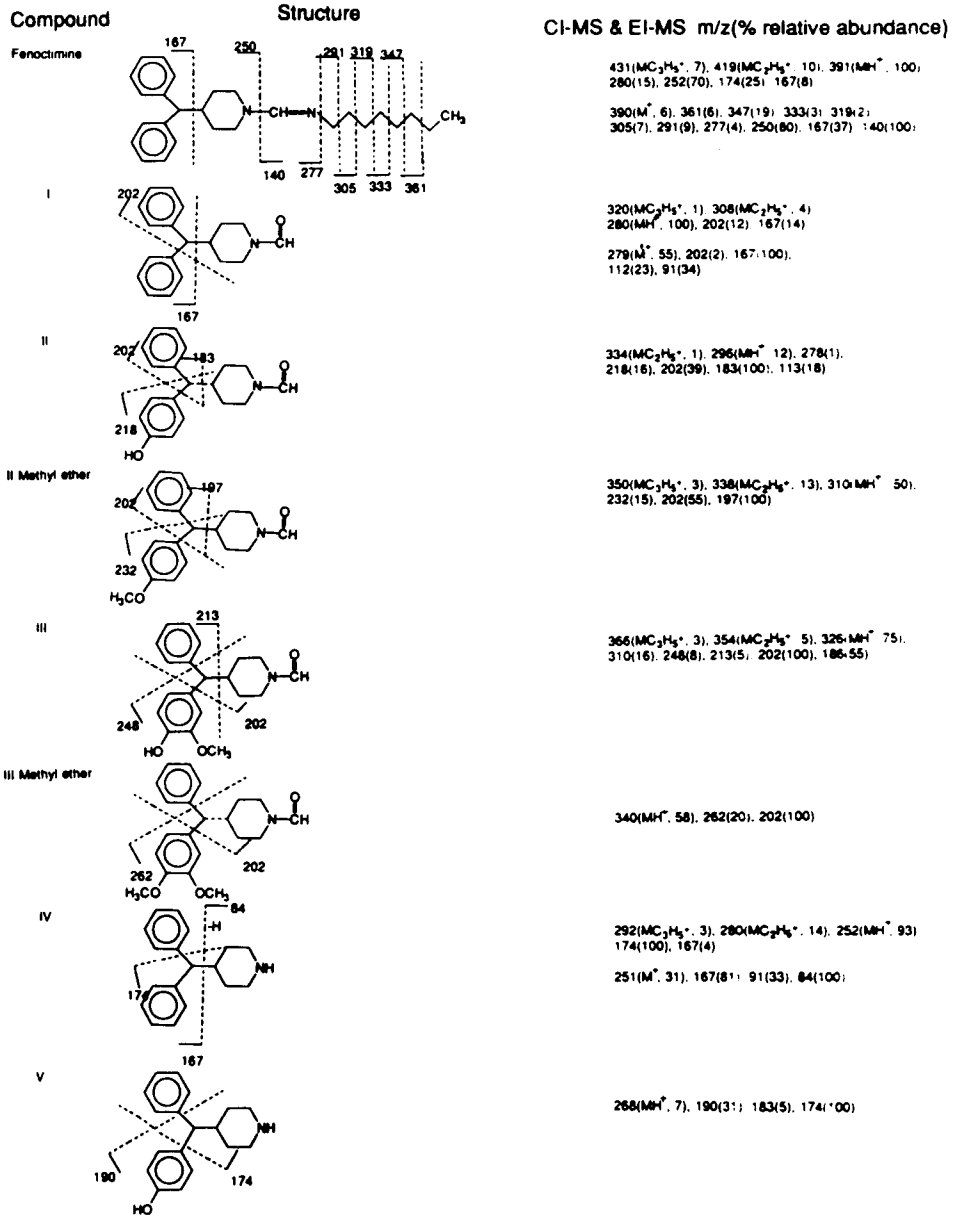

Figure 1. (*Continued*).

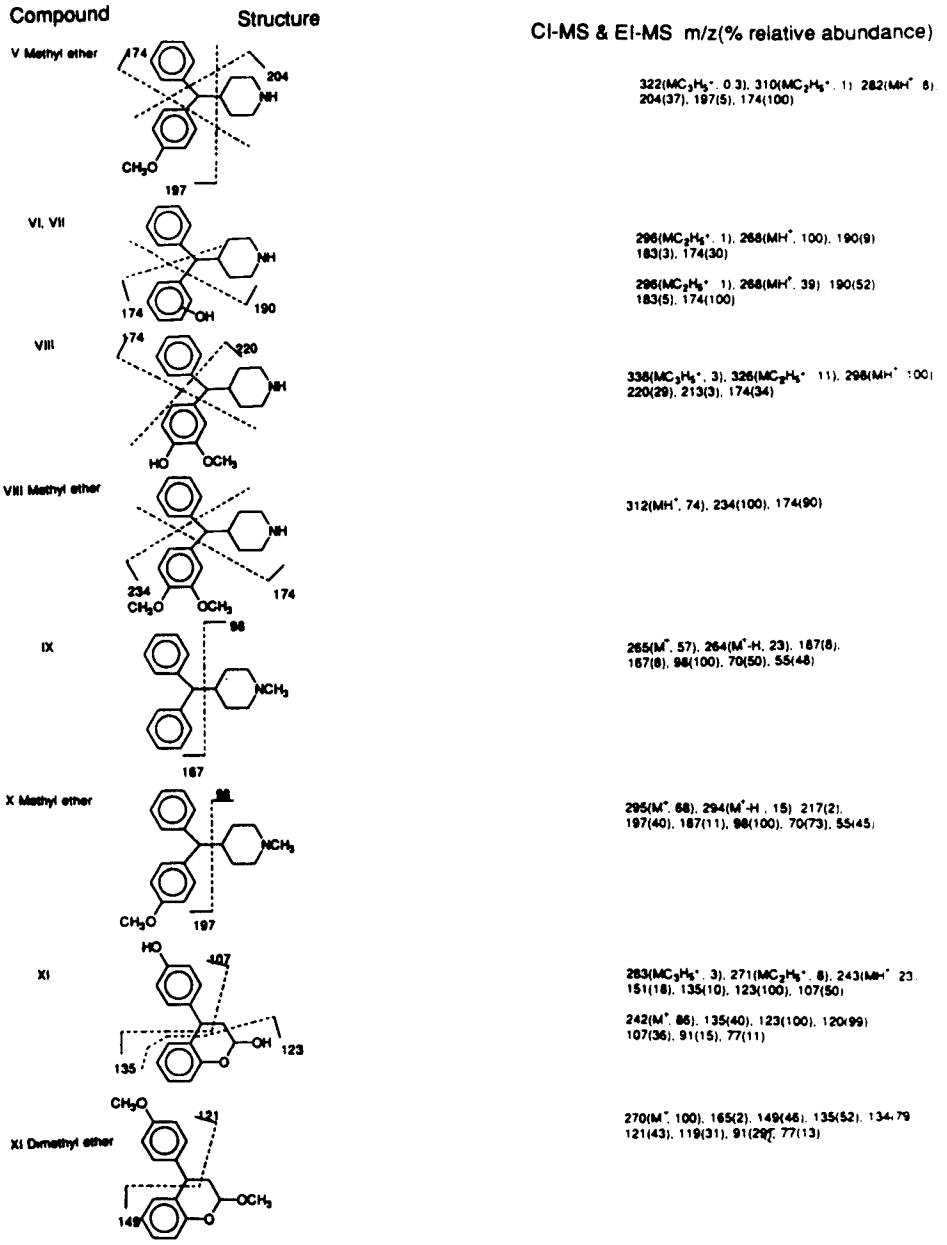

Figure 1. (*Continued*).

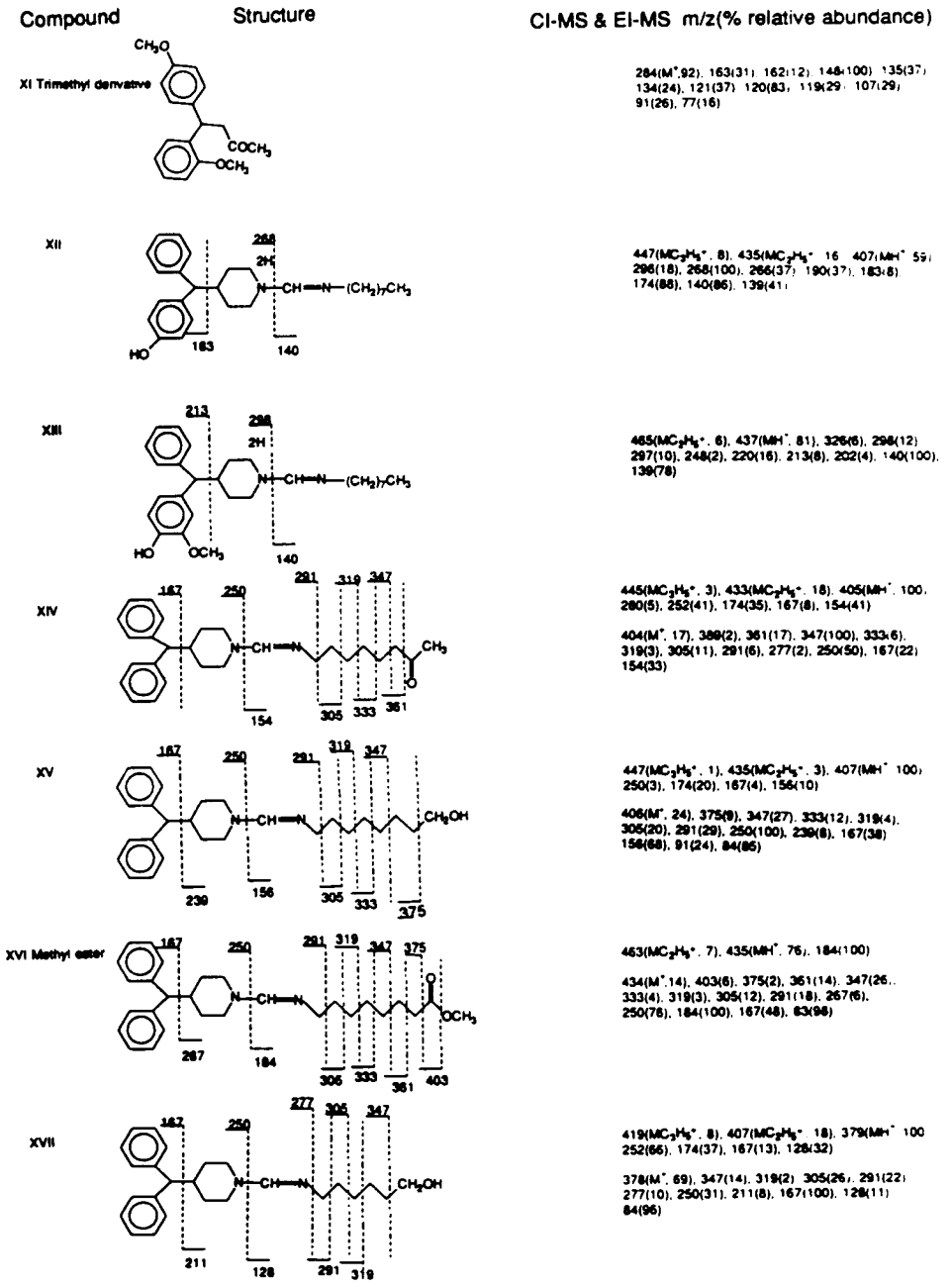

Figure 1. Chemical structures and mass spectral data for fenoctimine, its metabolites, and the derivatives.

Table 2. Tlc characteristics of fenoctimine metabolites and their derivatives.

Compound	R_f Silica gel GF 250 μm plates: ethyl acetate/isopropanol/ conc. ammonium hydroxide (80/20/2 v/v)
Fenoctimine	0·54–0·60
I	0·85–0·90
II	0·80–0·86
III	0·80–0·86
IV	0·15–0·20
V	0·10–0·15
VI	0·35–0·40
VII	0·32–0·35
VIII	0·15–0·20
IX	0·80–0·85
X Me ether	0·80–0·85
XI	0·75–0·80
XII	0·30–0·36
XIII	0·25–0·30
XIV	0·30–0·36
XV	0·40–0·44
XVI	0–0·05
XVI Me ester	0·34–0·40

Table 3. Hplc retention times for fenoctimine metabolites and their derivatives.

Compound	Hplc condition A R_t(min)	Hplc condition B R_t(min)
Fenoctimine	7·4–8·0	13·1
I	1·8–2·0	1·4
II	1·5–2·0	—
III	1·5–2·0	—
IV	3·6–4·0	6·7
V	3·0–3·5	3·0
VI	4·0–4·5	—
VII	4·5–5·0	—
VIII	3·0–3·5	—
XI	3·0–3·5	2·0
XII	5·6–6·6	11·2
XIII	5·5–6·5	—
XIV	5·6–6·6	not present
XV	—	18·6
XVII	—	11·6
McN-4978 (RWJ-34978)	5·3–6·3	12·6

Table 4. Percentages of total sample and dose from rat, dog and man as fenoctimine metabolites.

		Rat		Dog		Man	
Metabolite	Source	% Sample	(% Dose)	% Sample	(% Dose)	% Sample	(% Dose)
Fenoctimine	Bile	< 1					
	Plasma						
	Faeces					< 1	
I	Bile			6	(1·1)		
	Urine			< 1		< 1	
	Faeces					9·6	(4·1)
	Plasma					< 1	
II	Bile	6	(1·4)	6	(1·1)		
	Urine			< 1		< 1	
	Faeces					< 1	
III	Bile	6	(1·4)	< 1			
	Urine			< 1			
IV	Bile			< 1			
	Urine			< 1		32	(7·9)
	Faeces					15	(6·5)
	Plasma					> 50	
V	Bile	20	(4·6)	20	(3·7)		
	Urine			< 1		28	(6·9)
	Faeces					8	(3·4)
VI	Bile	< 1					
	Urine			< 1			
VII	Urine			33	(1·1)	9	(2·2)
VIII	Bile	< 1		< 1			
	Urine			< 1			
IX	Faeces					4·5	(1·9)
X	Faeces					< 1	
XI	Bile	40	(9·2)				
XII	Bile	< 1		25	(4·6)		
XIII	Bile			20	(3·7)		
XIV	Bile			< 1			
XV	Urine					12·3	(3·0)
	Faeces					11·7	(5·0)
XVI	Faeces					20·4	(8·8)
XVII	Urine					7	(1·7)
Total	Bile	75	(17)	80	(15)		
identified	Urine			40	(1)	90	20
metabolites	Faeces					70	30
	Plasma					50	

intense adduct molecular ion at m/z 296 along with several significant ions at m/z 278 (MH^+-H_2O) 218, 202, 183 (100%) and 113, which indicate a hydroxy group attached to a phenyl ring. High resolution EI-MS demonstrated an intense molecular ion with an accurate mass measurement of m/z 295·1569 indicating a composition of $C_{19}H_{21}NO_2$ (theoretical mass 295·1571). Other prominent ions were similar to those observed in CI. These data were consistent with the structure proposed.

A methyl ether derivative was obtained after treatment of **II** with diazomethane. The CI-MS of this product (figure 1) revealed an intense protonated molecular ion at m/z 310 (14 amu higher than **II**) and three prominent fragment ions at m/z 232, 202 and 197 (100%). This was further support for the proposed structure of **II**. Metabolite **II** was also obtained as a major degradation product of 4-hydroxyphenyl fenoctimine after the treatment of fenoctimine with the NaOH aqueous solution. The assignment of the 4-position for the hydroxy group was based on the identical hplc, tlc and MS data of the degradation product and **II**.

Metabolite **III** was obtained from rat and dog bile, and dog urine. The isolated material gave a positive test for a phenol on tlc using phosphomolybdic acid. CI-MS analysis (figure 1) exhibited an intense adduct molecular ion at m/z 326 (75%) and several prominent ions at m/z 310 (MH^+-CH_4), 248, 213 and 202 (100%), which indicates that both hydroxy and methoxy groups are attached to the same phenyl ring. High resolution EI-MS produced a molecular ion at mass 325·1687 indicative of $C_{20}H_{23}NO_3$ (theoretical mass 325·1676) along with fragments similar to those observed after CI-MS analysis.

The CI-MS spectrum of the methyl ether derivative **III** (figure 1) indicated an intense protonated molecular ion at m/z 340, 14 amu higher than the parent compound along with two key fragment ions at m/z 262 and 202 (100%). These results suggest that the two methoxy groups are attached to the same phenyl ring. The proposed positions of the functional groups are based on known biotransformation pathways concerning similar substitutions on structures, e.g. catecholamines (Testa *et al.* 1976) and meperidine (Yeh *et al.* 1981).

Metabolite **IV** appeared to be a major metabolite in human plasma, urine and faecal extracts, representing >50, 32 and 15% of the total radioactivity in the respective samples. A trace amount was also isolated from dog bile. The CI-MS analysis of the isolated material indicated an intense protonated molecular ion at m/z 252 (93·1%) and two prominent ions at m/z 174 (100%) and 167. High resolution EI-MS revealed an apparent molecular ion at mass 251·1669, which is consistent with an empirical formula of $C_{18}H_{21}N$ (theoretical mass 251·1673) and two intense ions at m/z 167 and 84 (100%). The identity of the isolated material was confirmed by comparison with an authentic sample.

Metabolite **V** was isolated in substantial amounts from rat and dog bile and human urine. Small quantities were also obtained from human faecal extracts and dog urine. Analysis of the isolated metabolite by CI-MS (figure 1) showed an apparent adduct molecular ion at m/z 268 and three significant fragment ions at m/z 190, 183 and 174 (100%). These results suggest that the hydroxy group is attached to the phenyl ring of the molecule. The CI-MS of the methyl ether of **V** (figure 1) gave a protonated molecular ion at m/z 282 (14 amu higher than that of **V**) together with the three major ions at m/z 204, 197 and 174 (100%). These MS data were consistent with the proposed structure. The ^1H nmr spectrum of the methyl ether (table 5) contained a singlet at δ3·80 indicative of a methoxy group, an AA'BB' quarter centred at δ6·80 and 7·20 for the four aromatic protons on the methoxylated

Table 5. ^{1}H nmr spectral data for fenoctimine and selected metabolites.

Compound	Phenyl protons	Chemical shifts (δ) CH_3O	$N-CH_3$	Aliphatic H's
Fenoctimine	6·80–7·30 (m) 10 H's			0·60–4·00 (m) 27 H's
V methyl ether	6·60 (d), 7·25 (d) 4 H's $J = 7$ Hz 7·20 ~ 7·60 (m) 5 H's	3·80 (s)		0·80–3·60 (m) 10 H's
X methyl ether	6·77 (d) 7·20 (d) 4 H's $J = 7$ Hz 7·23 ~ 7·50 (m) 5 H's	3·85 (s)	2·22	0·90 ~ 3·60 (m) 10 H's
XII	6·74 (d), 7·13 (d) 4 H's $J = 7$ Hz 7·15–7·30 (m) 5 H's			

ring, and a multiplet at δ7·40–7·45 attributed to the five aromatic protons of the other phenyl ring. Direct comparison of the chromatographic (tlc and hplc) and the spectral data from the methyl ether with that obtained from an authentic sample confirmed the identity of **V**.

Metabolites **VI** and **VII** were isolated from rat bile (**VI**), dog urine (**VI, VII**) and human urine (**VII**). Metabolite **VI** was present in small quantities in rat bile and dog urine, whereas **VII** was a major component of dog and human urine. The tlc and hplc behaviour of **VI** and **VII** were significantly different from that of **V** and from each other, even though their CI-MS (figure 1) were very similar to that obtained for **V**. The structures of metabolite **VI** and **VII** were uncertain and tentatively proposed as either 5-OH or 6-OH phenyl analogues on the basis of their tlc, hplc and CI-MS data. They are positional isomers of **V**.

Metabolite **VIII** was isolated in trace amounts from rat bile, and dog bile and urine. CI-MS of the isolated material (figure 1) revealed an apparent protonated molecular ion at m/z 298 (100%). Three additional fragment ions at m/z 220, 213 and 174 indicate that both hydroxy and methoxy groups are attached to the same phenyl ring. The metabolite was converted to the dimethyl ether, which was analysed by CI-MS (figure 1). The resulting spectrum consisted of intense ions at m/z 312 (MH^+), 234 and 174 indicative of the net addition of 14 amu to the parent compound. The proposed positions of the functional groups are based on documented biotransformation pathways concerning similar substitutions on structures, e.g. catecholamines (Testa *et al.* 1976) and meperidine (Yeh *et al.* 1981).

Trace amounts of **IX** and **X** methyl ether were isolated only as a mixture from human faecal extracts after diazomethane treatment. The CI-MS of the mixture exhibited two intense protonated molecular ions at m/z 296 and 266. Subsequent analysis by high resolution EI-MS produced an apparent molecular ion at mass 265·1820 for **IX** $C_{19}H_{23}N$ (theoretical 265·1830) and at m/z 295·1958 for **X** methyl ether $C_{20}H_{25}NO$ (theoretical mass 295·1938). The remaining fragment ions observed in EI-MS agreed with the proposed structures if the m/z 187 and 167 ions were associated with **IX** and those at m/z 197 and 187 were associated with the methyl ether of **X**. Ions at m/z 98 (100%), 70 and 55 appeared to be from the piperidine ring of both compounds. The proposed **IX** and **X** methyl ether were synthesized by reacting 4-(diphenylmethyl) piperidine and 4-(4-methoxyphenyl, phenylmethyl)

piperidine, respectively, with sodium borohydride and formaldehyde in methanol solution. Comparison of the tlc, CI-MS and EI-MS data for the synthetic compounds with those of the isolated material indicated their identity. The ^{1}H nmr analysis (table 5) provide the final determination of the structure of **X** methyl ether.

Metabolite **XI** was a major component of the glusulase-treated rat bile. The CI-MS (figure 1) indicated intense ions at 243 (MH^+), 151, 135, 123 (100%) and 107, which were consistent with the proposed structure. The high resolution EI-MS also gave an intense molecular ion mass 242·0935, which was in good agreement with a formula of $C_{15}H_{14}O_3$ (theoretical 242·0934). Additional ions that supported the proposed structure occurred at m/z 135, 123 (100%), 120, 107, 91 and 77. The ^{1}H nmr analysis of **XI** indicated a hydroxy group attached to the 4-position of a phenyl ring of the molecule. A tlc spot test with phosphomolybdic acid for the metabolite was positive for a phenol (data not shown). Diazomethane treatment of the isolated material produced two products, a dimethyl ether (M^+, 270) and a trimethyl ether ($M^{+\cdot}$, 284), confirmed by the EI-MS analysis (figure 1). TMS and acetate derivatives were made of the isolated metabolite and the EI-MS analysis of each product indicated that the products were di-TMS ($M^{+\cdot}$, 426) and diacetates ($M^{+\cdot}$, 326) of **XI**. Additionally, **XI** could not be reduced by sodium borohydride.

Metabolite **XII**, was isolated in trace amounts from rat bile and in large quantities from dog bile. The CI-MS (figure 1) demonstrated an intense MH^+ ion at m/z 407 along with two adduct ions, $MC_3H_5^+$ and $MC_2H_5^+$ at m/z 447 and 435 respectively. Remaining prominent fragment ions at m/z 268 (100%), 190, 183 and 174 were indicative of a phenolic group attached to the fenoctimine molecule. The ^{1}H nmr spectra of the isolated metabolites are shown in table 5. Hydroxylation of the phenyl ring of fenoctimine at the 4-position was indicated by the appearance of two pairs of aromatic protons as AA'BB' quartets centred at δ6·74 and 7·13 (table 5). Comparison of the chromatographic (tlc and hplc) and the spectroscopic (nmr and MS) data of the isolated metabolite with those of a synthetic sample confirmed the identity.

Metabolite **XIII** was also isolated as a major product from dog bile. The CI-MS of the isolated material (figure 1) showed an apparent MH^+ at m/z 437 (80·8%) with another adduct ion, $MC_2H_5^+$ at m/z 465. The most prominent ions at m/z 140 (100%) and 139 (78·4%) were suggestive of no substitution at the aliphatic side chain. Remaining fragment ions further supported the proposed structure, particularly the fragments at m/z 248, 220 and 213, which indicate that both phenolic and methoxy groups are attached to the same phenyl ring of the compound. An acetate and a methoxy derivative of the metabolite were prepared and CI-MS analyses indicated that a CH_3CO (MH^+ : 479) or a methyl group (MH^+ : 451) was added to the parent compound. The proposed position of the functional groups are based on literature results with model compounds (Testa *et al.* 1976, Yeh *et al.* 1981).

Metabolite **XIV** was isolated in trace amounts from dog bile. The compound was identified by comparison of tlc R_f, hplc R_t and MS data of the metabolite with those of a synthetic sample (Scott *et al.* 1987). The CI-MS and EI-MS of the isolated product exhibited an intense adduct molecular ion at m/z 405 (100%) and an apparent molecular ion at m/z 404 respectively (figure 1). The prominent ions indicated in figure 1 clearly support the assigned position of the ketone group.

Metabolite **XV** was present in substantial quantities in human urine and faecal extracts. The CI- and EI-MS data of the product (figure 1) contained an intense adduct molecular ion and an apparent molecular ion at m/z 407 (100%) and 406,

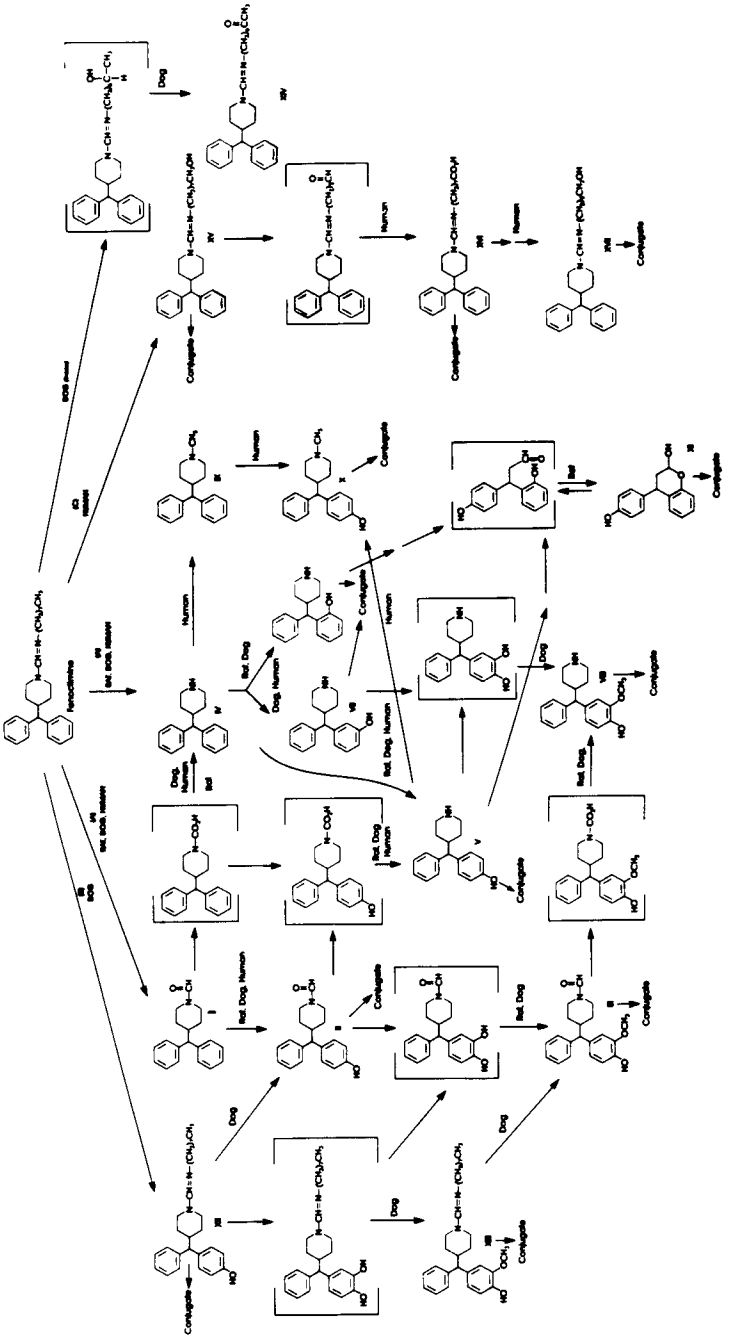

Figure 2. Proposed metabolic pathways for fenoctimine in rat, dog, and man.

respectively. Two prominent ions at m/z 375 (EI) and 156 (CI and EI) indicated a ω-hydroxy substitution on the aliphatic side chain of the molecule, whereas two other prominent ions at m/z 250 (EI, 100%) and 167 (EI and CI) revealed no substitution at the other moiety of the compound. The chromatographic (tlc and hplc) and the MS (CI and EI) data of the isolated metabolite and the synthetic product were identical (Scott *et al.* 1987).

Metabolite **XVI** was a major component of the human faecal extracts. The EI- and CI-MS of the methyl ester derivative (figure 1) showed an intense molecular ion and an apparent protonated molecular ion at m/z 434 and 435 respectively. The two important fragment ions at m/z 375 and 184 (100%) in EI clearly helped assign the position of the carboxyl group. The tlc characteristics and the CI- and EI-MS of the isolated metabolite and the synthetic product were identical (Scott *et al.* 1987).

Metabolite **XVII** was isolated from human urine. The EI-MS of the urinary metabolite (figure 1) showed an apparent molecular ion at m/z 378, which was further supported by an intense MH^+ at m/z 379 (100%). The fragmentation pattern obtained from EI-MS clearly assigned the proposed structure of **XVII**.

Discussion

Three metabolic pathways are proposed for fenoctimine in rat, dog and human, and are presented in figures 2 and 3.

In rat (figure 2), the biotransformation of fenoctimine appears to proceed through imino-bond cleavage to give **I** followed by further oxidation and decarboxylation to form **IV**. Subsequent aromatic oxidation results in the major metabolic products **II**, **III** and **V**. Metabolite **III** is a monomethylated form of the dihydroxylated intermediate. This type of metabolite has previously been reported for catecholamines (Testa *et al.* 1976) and meperidine (Yeh *et al.* 1981). Metabolites **V** and **VI** were further oxidized and cyclized to form a benzopyran analogue (**XI**), which was a major biliary metabolite.

In dog, the imino-bond cleavage pathway described above for rat is again prominent (figure 2). Both **I** and **IV** are present in bile and urine, with **I** representing 6% of the total radioactivity in the bile sample. Subsequent aromatic oxidation of

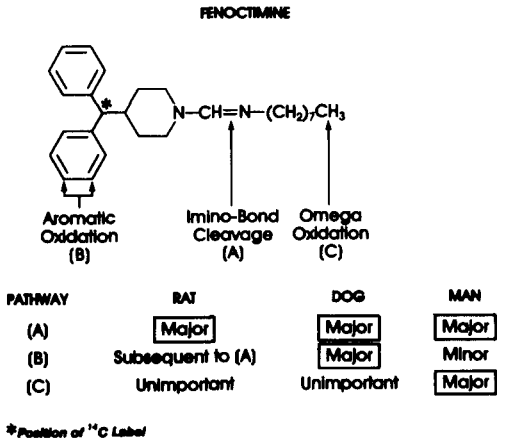

Figure 3. Quantitative importance of metabolic pathways of fenoctimine in rat, dog, and man.

I and **IV** results in two major biliary metabolites (**II** and **V**) and one urinary metabolite (**VII**, 33%). Interestingly, these biliary metabolites in dog represent the same percentages of total radioactivity in the sample as those observed in rat. However, the major benzopyran metabolite (**XI**) seen in rat bile was not found in dog bile or urine. An additional major biotransformation pathway was observed in dog bile samples. Aromatic oxidation resulted in **XII** (25%) and **XIII** (20%). It is possible that in dog this is the dominant pathway, as both biliary metabolites **II** and **V** can be produced from **XII** by hydrolysis.

In man (figure 2), imino-bond cleavage also appears to be the dominant pathway. The resulting **I** and **IV** are present in substantial quantities. Metabolite **IV** is a major component of total radioactivity in plasma (> 50%), urine (32%) and faecal extract samples (15%). Subsequent oxidation of **IV**, and possibly of **I**, produced three urinary metabolites (**II**, < 1; **V**, 28; and **VII**, 9%) and two faecal metabolites (**II**, < 1; and **V**, 8%). The *N*-methylated faecal metabolites (**IX** and **X**) could be produced by a pathway similar to that observed for normorphine, pyridine and nicotinic acid (Testa *et al.* 1976). It appears that, unlike rat and dog, oxidation in man does not progress past the formation of monohydroxylated aromatic metabolites. A second biotransformation pathway was observed only in man. This pathway proceeds by omega and further oxidation of the aliphatic chain and results in two urinary metabolites (**XV**, 12; and **XVII**, 7%) and two faecal metabolites (**XV**, 12; and **XVI**, 20%).

Metabolites **I** and **IV**, two major metabolic products for all species, showed no gastric antisecretory activity or anticholinergic activity. Metabolites **XII** and **XIV** were not anticholinergic, but they retained gastric antisecretory activity. Metabolites **XV**, **XVI** and **XVII** were 4–10 times less active antisectretory agents in dog than Fn (Scott *et al.* 1987).

In summary, three metabolic pathways for Fn were observed in rat, dog and human. An imino-bond cleavage pathway is important in all species. However, the other two pathways have differing importance in each species. An aromatic oxidation pathway could be the most important means of biotransformation of Fn in dog since all but two of the major metabolites can be formed by this route. The omega oxidation pathway, which produces three major metabolites, was observed in man, but not in the two animal species investigated.

In general, metabolism of Fn in all species appears to be a prototype of pathways from a combination of caecholamine-type oxidation at the phenyl ring, fatty acid oxidation at the side chain, and hydrolysis/cleavage.

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