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Human moricizine metabolism. I. Isolation and identification of metabolites in human urine

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1. Using synthetic standards and/or spectral data, seven moricizine metabolites were structurally identified in human urine. Two novel metabolites were identified as phenothiazine-2-carbamic acid and ethyl [10-(3-aminopropionyl) phenothiazin-2-yl] carbamate. Two novel human moricizine metabolites, 2-amino-10-(3-morpholinopropionyl) phenothiazine, a previously identified dog metabolite, and 2-aminophenothiazine, a previously identified rat metabolite, were also identified. Three additional human metabolites, phenothiazine-2-carbamic acid ethyl ester sulphoxide (P2CAEES), moricizine sulphoxide, and ethyl {10-[*N*-(2′-hydroxyethyl)3-aminopropionyl] phenothiazin-2-yl} carbamate, all previously described in the literature, were observed.

2. Both 2-amino-10-(3-morpholinopropionyl) phenothiazine and ethyl [10-(3-aminopropionyl) phenothiazin-2-yl] carbamate, and possibly ethyl {10-[*N*-(2′-hydroxyethyl)3-aminopropionyl]phenothiazin-2-yl} carbamate, possess the structural characteristics thought to be necessary for class 1 antiarrhythmic activity.

Introduction

Moricizine HCl (USAN ; moracizine, INN, or Ethmozine, the proprietary name frequently found in existing literature) (figure 1) is currently marketed in the USA and Europe as a class 1 antiarrhythmic agent. Mammalian metabolism of this compound has been the object of a limited number of investigations over the past two decades (Vikhlyaev *et al*. 1977, Sheehan *et al*. 1985, Sheehan 1990, Yang and Chan 1995). In man, however, moricizine metabolism has not been fully characterized. In addition, there is a lack of correlation between plasma moricizine concentration and onset, offset and duration of antiarrhythmic activity (Fitton and Buckley 1990). One explanation for this discrepancy is that certain moricizine metabolites, due to their modified chemical structures, may possess more potent antiarrhythmic activity than parent moricizine.

Antiarrhythmic drugs are classified based on their electrocardiographic and electrophysiologic effects. Class 1 antiarrhythmic agents are further subclassified into three groups: fast (1B), intermediate (1A) and slow (1C), based on the onset and recovery kinetics of use-dependent block of the maximum rate of depolarization (V_{max}) of cardiac intracellular action potentials and changes in action potential

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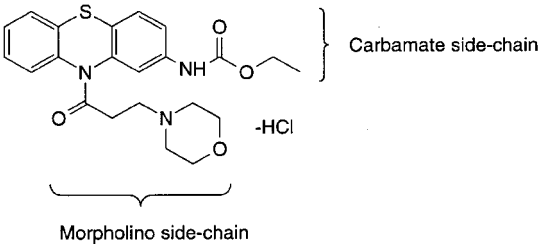


Figure 1. Moricizine HCl.

duration (APD) (Singh and Vaughan Williams 1970). This use-dependent block has been attributed to selective binding of drug to inactivated sodium channels or voltage-dependent binding (Hondeghem and Katzung 1977).

Moricizine has defied subclassification, having been described in the literature as a class 1A (Waldo *et al*. 1988), 1B (Snyder 1989), and 1C antiarrhythmic drug (Vaughan Williams 1992, Koller and Franz 1994). It has been suggested that the uniqueness of the antiarrhythmic behaviour of moricizine is due in part to the low pK_a (6·4) of the morpholine nitrogen (Schubert *et al*. 1986, Makielski *et al*. 1990). This pK_a is well below the physiological pH of 7·4, and therefore the drug exists largely as the lipophilic free base *in vivo*, although a small, changing fraction of the drug would be protonated. In this way, moricizine could exhibit a chameleon-like behaviour. In contrast, most class 1 antiarrhythmic drugs contain alkyl amines with much higher pK_a's (8–10), and exist largely as the charged ammonium ion species *in vivo*.

The morpholine nitrogen has a low pK_a relative to an alkyl amine because it has two bonds that are attached by a two-carbon bridge to the oxygen atom, each lowering the pK_a by approximately 1·1 pH units (Perrin *et al*. 1981). Therefore, any metabolism of the morpholine ring that results in loss of one or both of the two-carbon bridges between the morpholine nitrogen and oxygen results in a rise in the pK_a of the nitrogen. This paper describes the structural characterization of moricizine metabolites in human urine with particular emphasis on those metabolites that could potentially exhibit antiarrhythmic activity.

Materials and methods

Chemicals

Sodium metaperiodate, hydrogen peroxide, activated charcoal and reagent grade solvents were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Acrylonitrile, *t*-butylammonium hydrogen sulphate, benzyl chloroformate, morpholine, butyl lithium, potassium carbonate, magnesium sulphate, sulphuric acid, hydrochloric acid, sodium hydroxide and reagent grade solvents were obtained from Merck (Darmstadt, Germany). Analytical solvents and reagents: acetonitrile, methanol, acetic acid (J. T. Baker, Inc., Phillipsburg, NJ, USA), ammonium acetate, sodium 1-pentanesulphonate and sodium bicarbonate (Sigma Chemical Co., St Louis, MO, USA) were all of hplc or analytical grade. Deuterated water, acetone and DMSO were obtained from MSD isotopes (Dorval, Quebec, Canada). Water was purified using a Milli-Q system (Millipore Corp., Bedford, MA, USA).

Synthesis of reference compounds

A number of moricizine derivatives (including some known metabolites from animal studies) were synthesized as reference compounds for metabolic investigation. These included moricizine HCl, moricizine HCl sulphoxide, phenothiazine-2-carbamic acid ethyl ester sulphoxide, phenothiazine-2-carbamic acid, 2-aminophenothiazine and 2-amino-10-(3-morpholinopropionyl) phenothiazine. These compounds were used as authentic standards for the isolation of major metabolites by gradient hplc, and

for their identification by nmr and mass spectrometry. Depending on their chemical structure, they were prepared either from moricizine or phenothiazine-2-carbamic acid ethyl ester (P2CAEE) as starting materials (Gritsenko *et al.* 1972).

Moricizine HCl sulphoxide

Moricizine HCl sulphoxide was prepared by slowly adding a cold aqueous solution of moricizine HCl to a cold aqueous solution of sodium metaperiodate. The solution was stirred at 0–5 °C until the starting material had disappeared. The reaction mixture was extracted with methylene chloride, which was then stripped and the remaining solids dissolved in refluxing ethanol. After hot filtration, the filtrate was acidified with HCl to a pH of ~ 3·0 and stirred at room temperature until crystals appeared. After filtration, the solids were washed with cold ethanol and dried in a 60 °C vacuum oven. ^{1}H-nmr (D_2O): 1·2 (t, 3H), 3·15 (t, 2H), 3·2 (s, 4H), 3·4 (t, 2H), 3·8 (s, 4H), 4·18 (q, 2H), 7·2–7·9 (m, 7H). Ms (NH_3-DCI) : $(M+H)^+$, m/z = 444. Purity = ~ 93% by hplc.

Phenothiazine-2-carbamic acid ethyl ester (P2CAEE) sulphoxide

P2CAEE sulphoxide was prepared by dissolving P2CAEE in acetone and slowly adding 30% H_2O_2 at room temperature until all of the starting material had reacted. The reaction mixture was then stripped down and the solids dissolved in DMSO. After stirring with activated charcoal, the solution was filtered and the product solids precipitated from the DMSO by water addition. After cooling and filtration, the solids were stirred in refluxing ethanol. The solids were again filtered, ground and dried overnight in a 110 °C vacuum oven. M.P. 300 °C. ^{1}H-nmr (DMSO-d_6): 1·25 (t, 3H), 4·2 (q, 2H), 7·2–7·9 (m, 7H). Ms (NH_3-DCI) : $(M+H)^+$, m/z = 303. Purity = 99·3% by hplc.

Phenothiazine-2-carbamic acid and 2-amino phenothiazine

Phenothiazine-2-carbamic acid was prepared by adding 1 molar equivalent of NaOH to a solution of P2CAEE (5·3 g, 18·5 mmol) in ethanol (95%, 110 ml), under a nitrogen atmosphere. The mixture was refluxed for 1·5 h. The resulting precipitate was filtered and washed with ether. ^{1}H-nmr (D_2O): 6·64 (d, 1H), 6·70 (d of d, 1H), 7·25 (t, 1H), 7·36 (d, 1H), 7·65 (t, 1H), 7·72 (d, 1H), 7·92 (d, 1H), 8·44 (s, 1H). This compound was decarboxylated to 2-aminophenothiazine by suspending it in water and neutralizing with 1 M HCl to pH 7. Carbon dioxide gas evolved during neutralization. A colour change was observed from olive green to purple. The resulting solid was dried on a vacuum dessicator to give 2-aminophenothiazine (3·5 g, 83%), m.p. 182–4 °C. ^{1}H-nmr (acetone-d_6): 2·8 (NH_2, 2H), 6·13 (s, 1H), 6·18 (d, 1H), 6·62 (d, 1H), 6·69 (d, 1H), 6·74 (t, 1H), 6·92 (d, 1H), 6·95 (t, 1H), 7·58 (NH, 1H). Ms (NH_3-DCI) : $(M+H)^+$, m/z = 215. Elemental analysis : C, 66·98 ; H, 4·92 ; N, 13·43 ; S, 14·76% ; $C_{12}H_{10}N_2S$ requires C, 67·26 ; H, 4·7 ; N, 13·07 ; S, 14·96%.

2-Amino-10-(3-morpholinopropionyl) phenothiazine

2-Amino-10-(3-morpholinopropionyl) phenothiazine was prepared from 2-aminophenothiazine by protection of the amino group to give phenothiazine-2-carbamic acid benzyl ester (P2CABE), followed by addition to the morpholino side chain and subsequent deprotection. To a solution of 2-aminophenothiazine (3·9 g, 19·3 mmol) in 60 ml methylene chloride were added NaOH (0·95 g, 23·9 mmol), 15 ml water and tetrabutylammonium hydrogen sulphate (5 mg). The mixture was cooled under nitrogen to 10 °C and a solution of benzylchloroformate (6·6 ml, 19·9 mmol) in toluene (50% was added dropwise. After 15 min the organic layer was separated and the aqueous layer was extracted with methylene chloride. The organic layer was washed with 1 M HCl, dried over $MgSO_4$ and evaporated under reduced pressure. The resulting concentrate was chromatographed on silica gel (eluent = 1 : 1 hexane : ethyl acetate) to yield P2CABE (2·4 g, 47%). ^{1}H-nmr (DMSO-d_6): 5·12 (s, 2H), 6·63–6·92 (m, 7H), 7·30–7·36 (m, 5H), 8·11 (s, 1H), 9·15 (s, 1H). Elemental analysis : C, 69·05 ; H, 4·87 ; N, 8·09 ; S, 9·43 ; $C_{20}H_{16}O_2N_2S$ requires C, 68·95 ; H, 4·63 ; N, 8·04 ; S, 9·2%. The 3-morpholinopropionic acid ethyl ester was prepared by addition of morpholine to acrylonitrile (Whitmore *et al.* 1944), followed by reflux with ethanol in the presence of sulphuric acid (Weisel *et al.* 1945). Then, to a solution of P2CABE (1·09 g, 4 mmol) in 20 ml tetrahydrofuran under nitrogen at −65 °C, BuLi (5·27 ml, 1·4 M, 8 mmol) was added by a syringe. Deprotonation continued for 10 min at −65 °C. Then a solution of 3-morpholinopropionic acid ethyl ester (1·5 g, 8 mmol) in 6 ml dry tetrahydrofuran was added dropwise and reacted for 1 h at −65 °C, then 1 h at 25 °C. The reaction was quenched with 20 ml water, then extracted twice with diethyl ether and once with ethyl acetate. The combined organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was chromatographed on silica gel [eluent ; ethyl acetate : methanol (9 : 1)]. The product was recrystallized from ethyl acetate/hexane (1 : 9) to give the benzyl ester analogue of moricizine (0·413 g, 25%). This compound was treated with 40% HBr in 2 ml acetic acid at 25 °C. A colour change to purple was observed. After 0·5 h, 10 ml dry diethyl ether was added and the precipitate was cooled on a Buchner funnel under nitrogen, then was suspended in K_2CO_3

Table 1. Metabolite retention times on hplc systems 1–3.

Metabolite	System 1 *RT*[a] (min)		System 2 *RT* (min)		System 2a (pH 4·5) *RT* (min)	System 3[d] *RT* (min)	
	Urinary metabolite	Synthetic standard	Urinary metabolite	Synthetic standard	Urinary metabolite	Urinary metabolite	Synthetic standard
(Moricizine)	33·78	32·92	nd[b]	26·49	nd	nd	nd
1	10·35	10·79	23·08	23·05	nd	7·21	6·78
2	15·36	15·44	12·44	12·44	nd	18·30	18·28
3	23·08	23·32	nd	nd	nd	nd	nd
4	26·19	25·88	nd	22·45	nd	nd	nd
5	33·00	na[c]	28·53	na	24·07	6·60	na
6	33·00	na	28·53	na	24·98	24·10	na
7	35·41	34·69	nd	23·55	nd	nd	nd

[a] *RT*, retention time.
[b] nd, not determined.
[c] na, standard not available.
[d] Ratios of ammonium acetate buffer : methanol : water varied for different metabolites.

solution. The free amine was extracted into ethyl acetate. The organic layer was dried over $MgSO_4$ and evaporated to obtain 2-amino-10-(3-morpholinopropionyl) phenothiazine (0·23 g, 80%). ^{1}H-nmr (acetone-d_6), 2·35 (s, 2H), 2·6–2·9 (m, 10H), 6·6 (d, 1H), 6·95 (s, 1H), 7·15 (d, 1H), 7·25 (t, 1H), 7·30 (t, 1H), 7·42 (d, 1H), 7·6 (d, 1H). Ms (NH_3-DCI) : $(M+H)^+$, m/z = 356. Elemental analysis: C, 64·45; H, 6·23; N, 11·56; S, 9·23; $C_{19}H_{21}O_2N_3S$ requires C, 64·20; H, 5·95; N, 11·82; S, 9·02%.

Drug administration and sample collection

Healthy adult male and female subjects, between the ages of 19 and 30 years and weighing 50–86 kg, signed informed consent and were entered into the study, which was approved by the Clinical Research Institutional Review Committee (Needham, MA, USA) for Medical and Technical Research Associates (Boston, MA, USA). Following overnight fasts, each subject received a single, oral 250 mg dose of moricizine HCl. Urine collections were made at predose and during the 0–12-h interval post-dose. During each collection period, the urine was stored in refrigerated vessels. All samples were stored at $\leqslant -20\,^{\circ}C$ until analysed.

Instrumentation/conditions

Hplc equipment consisted of a Waters Associates (Milford, MA, USA) 600E pump and controller and a Waters WISP sample injector. Column temperature was controlled using a Dual Zone Systec (Thomson Instrument Co., Newark, DE, USA) temperature controller. Ultraviolet absorption was monitored with an ABI (Foster City, CA, USA) 783A programmable absorbance detector and a Hewlett-Packard (Wilmington, DE, USA) 3392A Integrator.

Metabolite samples (10–20 μg) and synthetic samples (1–2 mg) for nmr analysis were dissolved in deuterated water, DMSO or acetone. The spectra were obtained using a Varian VXR 400S, a Varian VXR 500 instrument (Palo Alto, CA, USA), or a Bruker AC-200E instrument (Karlsruhe, Germany). Chemical shifts are expressed in ppm, set relative to the solvent. Metabolites were isolated in the presence of ammonium acetate and therefore the samples exhibited a resonance at δ = 1·9 from the acetate ion in addition to the water resonance at δ = 4·7.

Low resolution mass spectra were obtained with a Finnigan MAT 8230 mass spectrometer (Bremen, Germany) using a direct exposure probe and ammonia chemical ionization (NH_3-DCI). The samples were deposited onto the probe from methanol/methylene chloride solutions.

Identification of urinary metabolites by chromatographic comparison with synthetic standards

Standards of known rat, dog and human moricizine metabolites (and their intermediates) were prepared synthetically and characterized by nmr, ms, and elemental analysis. The relative retention time (RRT) of each synthetic standard (versus moricizine) was compared with the relative retention times of human plasma or urine metabolites under specific hplc conditions (table 1).

RRT_{std} = retention time of standard/retention time of moricizine
RRT_{met} = retention time of metabolite/retention time of moricizine.

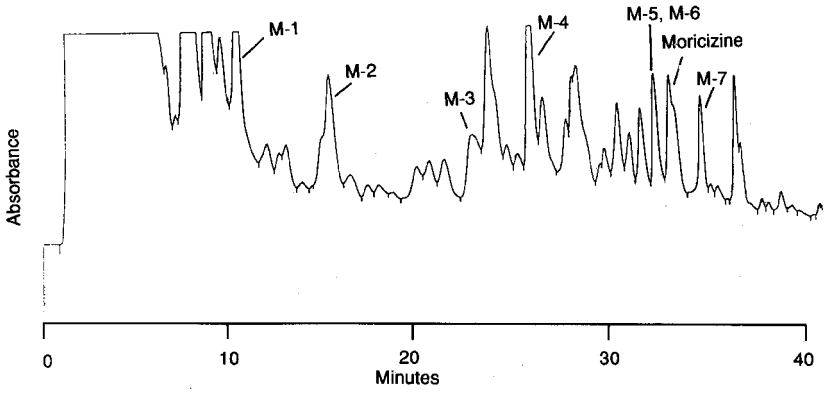


Figure 2. Representative hplc chromatogram (system 1) of a typical 0–12-h urine extract from a female volunteer containing moricizine and its metabolites.

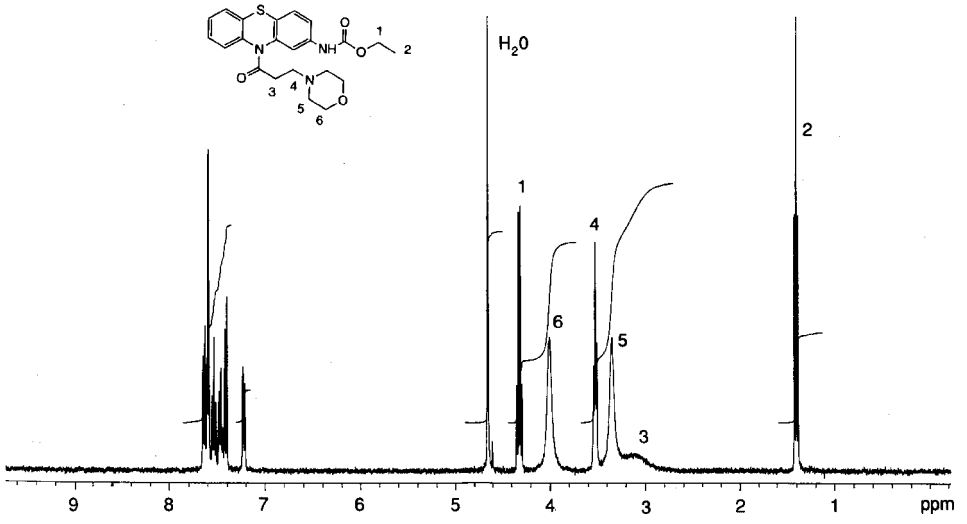


Figure 3. ^{1}H-nmr spectrum of moricizine HCl (approximately 1 mg in deuterated water).

Correlation was said to occur when the absolute value of

$$[RRT_{std} - RRT_{met}] < 0{\cdot}05.$$

Isolation of metabolites

The metabolites were isolated using a combination of solid phase extraction (s.p.e.) and hplc. Untreated urine (160 ml) was centrifuged and applied to a Mega BondElut (Varian Sample Preparation Products, Harbor City, CA, USA) C-18 s.p.e. column (pre-conditioned with 2-column vols methanol, then 1-column vol. water) using nitrogen pressure. The column was washed three times with 5 ml water, then eluted seven times with 5 ml methanol. The eluent was taken to dryness under nitrogen at 37 °C. The sample was reconstituted with hplc mobile phase and filtered with 0·45 μm Ultrafree filters (Millipore Corp., Bedford, MA, USA). Three to four sequential hplc purification steps were used. For hplc system 1, a chromegabond MC-18 (15 cm × 14 mm, 5 μm, 60A, ES Industries, Berlin, NJ, USA) column at 35 °C was used. S.p.e. fractions of urine were analysed using a linear gradient developed from 85 : 15 A : B to 10 : 90 A : B over 50 min (A = 90% H_2O, 10% MeOH, 2·5 mM sodium-pentanesulphonate, 0·1% H_3PO_4; B = 95% MeOH, 5% H_2O, 2·5 mM sodium-pentanesulphonate, 0·1% H_3PO_4) (figure 2). Metabolite fractions were collected from system 1, neutralized with sodium bicarbonate and dried under vacuum.

For hplc systems 2 and 2a, a Beckman Ultrasphere ODS (25 cm, 5 μm; Fullerton, CA, USA) column

at 35 °C was used. Fractions collected from system 1 were analysed using a linear gradient (varied for each metabolite) of A and B [A = 90% 1·0 M ammonium acetate (neutral or pH 4·5 for 2 and 2a respectively), 10% MeOH; B = 100% MeOH]. Analysis on system 2 was repeated if necessary.

For hplc system 3, a Beckman Ultrasphere ODS (25 cm, 5 μm) column was used. Metabolite fractions collected from system 2 were analysed using an isocratic elution of 7:27:66 (varied slightly for each metabolite) A:B:C [A = 90% 0·1 M ammonium acetate (neutral or pH 4·5), 10% MeOH; B = 100% MeOH; C = 90% H_2O, 10% MeOH]. Metabolites were detected at a wavelength of 254 nm.

Results

Identification of individual urinary metabolites

Metabolite 1. Synthetically prepared 2-amino-10-(3-morpholinopropionyl) phenothiazine, a previously described dog metabolite (K. W. Sigvardson, A. F. Davidson and H. J. Pieniaszek Jr, unpublished data), was shown to co-chromatograph with metabolite 1 on hplc systems 1–3. The NH_3-DCI mass spectra of both the standard and metabolite 1 (figure 4A) exhibited the $(M+H)^+$ species at m/z = 356 and little or no fragmentation.

Metabolite 2. The synthetically prepared sodium salt of phenothiazine-2-carbamic acid was shown to co-chromatograph with metabolite 2 on hplc systems 1–3. In addition, the ^{1}H-nmr of about 50 μg of metabolite 3 in D_2O [6·64 (d, 1H), 6·70 (d of d, 1H), 7·25 (t, 1H), 7·36 (d, 1H), 7·65 (t, 1H), 7·72 (d, 1H), 7·92 (d, 1H), 8·44 (s, 1H)] (figure 5) was identical to that of the synthetically prepared standard. When metabolite 2 was isolated and exposed to acidic conditions (i.e. evaporation to dryness in acidic mobile phase without prior neutralization), subsequent analysis showed a small amount of degradation to a compound identified as 2-aminophenothiazine (by hplc retention comparison with authentic standard). Presumably this was the result of acid-catalysed decarboxylation of the carbamic acid (analogous to the acid catalysed decarboxylation of synthetic phenothiazine-2-carbamic acid to give 2-aminophenothiazine described under Synthesis of reference compounds).

Metabolite 3. Synthetically prepared 2-aminophenothiazine, a previously described rat metabolite (Vikhlyaev *et al.* 1977), was shown to co-chromatograph with metabolite 3 on hplc system 1. These data are consistent with the presence of 2-aminophenothiazine in human urine, although unequivocal structural determination has yet to be completed.

Metabolite 4. Synthetically prepared moricizine sulphoxide, a previously described, pharmacologically active human metabolite (Sheehan 1990, Pieniaszek *et al.* 1994, Yang and Chan 1995), a rat metabolite (Vikhlyaev *et al.* 1977), and a dog metabolite (Sigvardson, Davidson and Pieniaszek Jr, unpublished data), was shown to co-chromatograph with metabolite 4 on hplc system 1. This metabolite has been well-characterized in human urine and therefore was not extensively analysed in the present study.

Metabolite 5. The ^{1}H-nmr spectrum of metabolite 5 was obtained in D_2O from about 12 μg isolated material [1·2 (t, 3H), 2·95 (t, 1H), 3·15 (t, 1H), 3·55 (t, 2H), 3·85 (m, 4H), 4·0 (m, 4H), 4·15 (q, 2H), 7·20 (d, 1H), 7·25 (t, 1H), 7·30 (t, 1H), 7·45 (d,

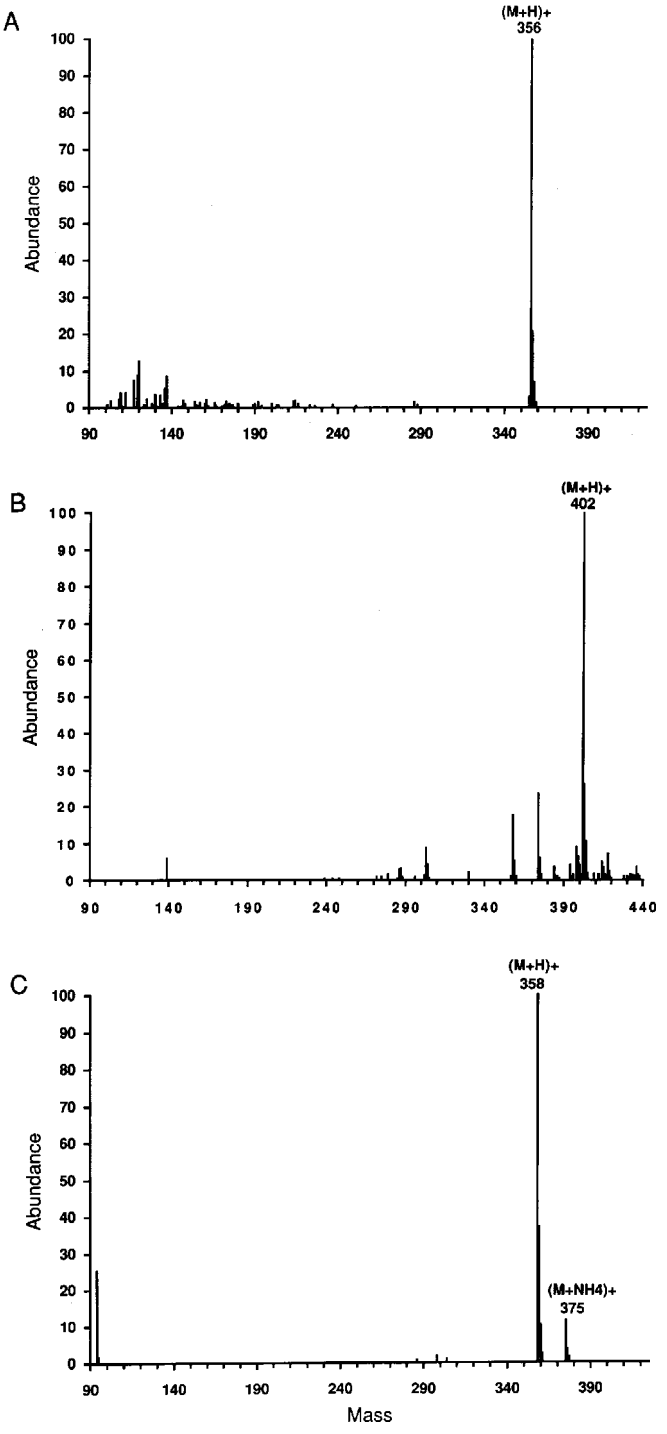


Figure 4. NH_3-DCl m.s. of (A) metabolite 1 [$(M+H)^+ = 356$], (B) metabolite 5 [$(M+H)^+ = 402$], and (C) metabolite 6 [$(M+H)^+ = 358$].

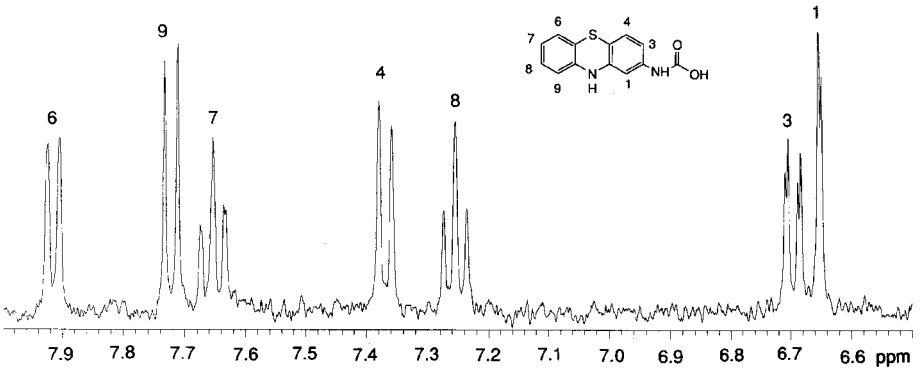


Figure 5. The aromatic region of the ^{1}H-nmr spectrum of metabolite 2 (approximately 20 μg in deuterated water).

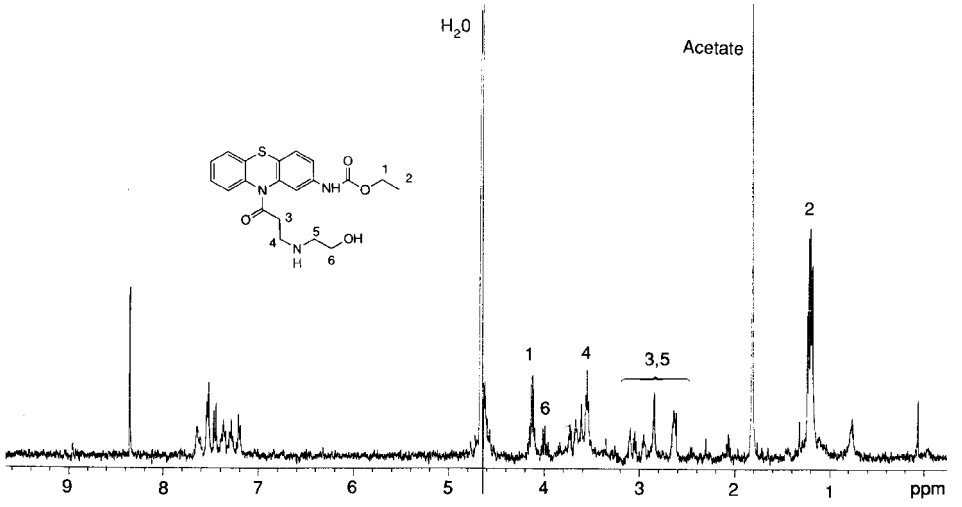


Figure 6. ^{1}H-nmr of metabolite 5 (approximately 12 μg in deuterated water).

1H), 7·50 (d, 2H), 7·65 (s, 1H), 8·35 (s, 1H)] (figure 6). This showed that the carbamate side chain of moricizine was intact but the morpholino side chain was structurally altered. The NH_3-DCI mass spectrum of metabolite 5 exhibited $(M+H)^+$ at m/z = 402 (figure 4B). These data are consistent with an *N*-2-hydroxyethyl group in place of the morpholino ring (i.e. 10[3-N-(2′-hydroxyethyl) amino propionyl] phenothiazin-2-carbamic acid ethyl ester), a metabolite structure that has previously been described in human urine (Sheehan *et al*. 1985).

Metabolite 6. The ^{1}H-nmr spectrum of metabolite 6 in D_2O was obtained from about 10 μg isolated material [1·2 (t, 3H), 3·1 (m, 1H), 3·2 (t, 2H), 3·3 (t, 1H), 4·2 (q, 2H), 7·22 (d, 1H), 7·35 (t, 1H), 7·42 (t, 1H), 7·5 (d, 1H), 7·58 (d, 2H), 7·7 (s, 1H), 8·4 (s, 1H) (figure 7). It showed that the carbamate side chain was intact. The proton resonances of the morpholino ring were absent but the resonances of the 3-aminopropionyl section were present. The NH_3-DCI mass spectrum exhibited $(M+H)^+$ at m/z = 358 (figure 4C). These data are consistent with a primary amine in place of the morpholino ring of moricizine (i.e., ethyl [10-(3-aminopropionyl) phenothiazin-2-yl] carbamate).

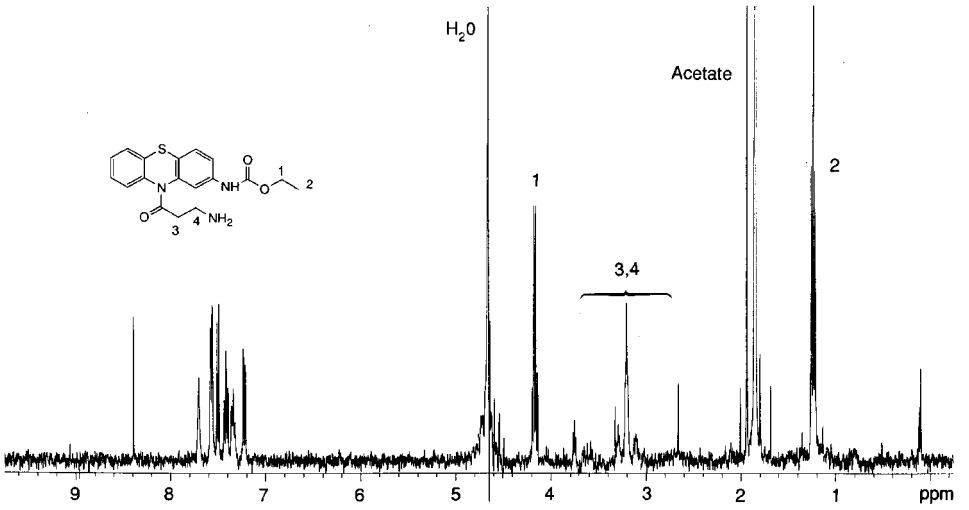


Figure 7. ^{1}H-nmr spectrum of metabolite 6 (approximately 10 μg in deuterated water).

Metabolite 7. Synthetically prepared phenothiazine-2-carbamic acid ethyl ester sulphoxide, a previously described human metabolite (Sheehan 1990), rat metabolite (Vikhlyaev *et al.* 1977) and dog metabolite (Sigvardson, Davidson and Pieniaszek Jr, unpublished data) was shown to co-chromatograph with metabolite 7 on hplc system 1. This metabolite has been well characterized in human urine and therefore was not extensively analysed in the present study.

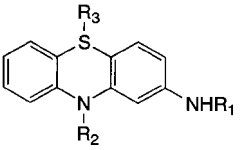
Discussion

Moricizine metabolites described herein (table 2) provide an outline of the metabolic pathways of this drug in man (figure 8). Many of the human urine metabolites of moricizine, although not structurally identified, were quantified in a separate study employing ^{14}C-moricizine (H. J. Pieniaszek, Jr., unpublished data. In this latter radiolabelled study, the combined percent of dose for all identified urinary metabolites was very small ($< 7\%$), although several of these metabolites achieved significant plasma concentrations and were eliminated with longer half-lives relative to parent drug. Additionally, the UV-detected chromatographic metabolic profile in plasma correlated closely with the urine profile.

Earlier investigators of human moricizine metabolism have described the 2-hydroxyethyl amine derivative (ethyl {10-[*N*-(2-hydroxyethyl)3-aminopropionyl] phenothiazin-2-yl} carbamate), the sulphoxide of the bis-2-hydroxyethylamine derivative (ethyl {10-[*N*,*N*-(2-hydroxyethyl)3-aminopropionyl] phenothiazin-2-yl} carbamate sulphoxide), ring-hydroxylated phenothiazine-2-carbamic acid ethyl ester, 2-acetamido-phenothiazine sulphoxide, 10-(3-acetylaminopropionyl)-2-aminophenothiazine sulphoxide and 10-(3-propionylmorpholin-2-one)-2-aminophenothiazine sulphoxide (Sheehan *et al.* 1985, Sheehan 1990). A recent report describes the identification of moricizine sulphone (Yang and Chan 1995). In addition, a glucuronide conjugate of 2-aminophenothiazine has been identified (L. Shum, unpublished data).

There appear to be three primary pathways of human biotransformation of moricizine: oxidation of the phenothiazine sulphur, hydrolysis or hydrolysis

Table 2. Structures of moricizine metabolites isolated from human urine.



Metabolite number	Compound	R_1	R_2	R_3
0	Moricizine	$CO_2CH_2CH_3$	$COCH_2CH_2N(CH_2CH_2)_2O$	–
1	2-amino-10-(3-morpholinopropionyl)-phenothiazine[a]	H	$COCH_2CH_2N(CH_2CH_2)_2O$	–
2	phenothiazine-2-carbamic acid	CO_2H	H	–
3	2-aminophenothiazine[b]	H	H	–
4	moricizine sulphoxide[a,b]	$CO_2CH_2CH_3$	$COCH_2CH_2N(CH_2CH_2)_2O$	O
5	ethyl (10-[N-(2′-hydroxyethyl) 3-aminopropionyl] phenothiazin-2-yl) carbamate	$CO_2CH_2CH_3$	$COCH_2CH_2NHCH_2CH_2OH$	–
6	ethyl [10-(3-aminopropionyl) phenothiazin-2-yl] carbamate	$CO_2CH_2CH_3$	$COCH_2CH_2NH_2$	–
7	phenothiazin-2-carbamic acid ethyl ester sulphoxide[a,b]	$CO_2CH_2CH_3$	H	O

[a] Previously identified as a dog metabolite (K. W. Sigvardson, A. F. Davidson and H. J. Pieniaszek Jr, unpublished data).
[b] Previously reported rat metabolite (Vikhlyaev et al. 1977).

followed by decarboxylation of the carbamate side chains and oxidation and/or cleavage of the morpholino side chain. In addition, primary metabolites can undergo further biotransformation by another of the above reactions or undergo phase II metabolism to generate conjugated products (Vikhlyaev *et al*. 1977).

Biotransformations analogous to many of these have been reported for drugs with similar structures. Oxidation of phenothiazine drugs to the corresponding sulphoxide has been described frequently in the literature (Beckett *et al*. 1963, Aravagiri *et al*. 1984). Ethacizin, a moricizine analogue, has been shown to undergo hydrolysis of the carbamic acid ethyl ester to generate the free carbamic acid metabolite in man (Beloborodov *et al*. 1989). Hydrolysis of the morpholino ring to generate the bis-(2-hydroxyethyl) amine derivative or oxidation α to the morpholino oxygen followed by hydrolysis of the resulting ester has been described for molsidomine metabolism in animals and man (Wilson *et al*. 1987). Oxidative mono- or di-*N*-dealkylation of an analogous moricizine intermediate would generate the 2-hydroxyethylamine derivative (metabolite 5) (Sheehan *et al*. 1985), or the primary amine derivative (metabolite 6), respectively. Interestingly, di-*N*-dealkylation of ethacizin in man generates a metabolite structurally identical to metabolite 6 (Beloborodov *et al*. 1989).

Several of the moricizine metabolites described above (1, 3 and 4) have been tested for antiarrhythmic activity in isolated guinea pig atria (B. S. Brown and A. L. Cigan, unpublished data). Moricizine sulphoxide was found to be approximately five times less potent than moricizine. All other metabolites tested did not contain any part of the morpholine ring and did not exhibit any activity.

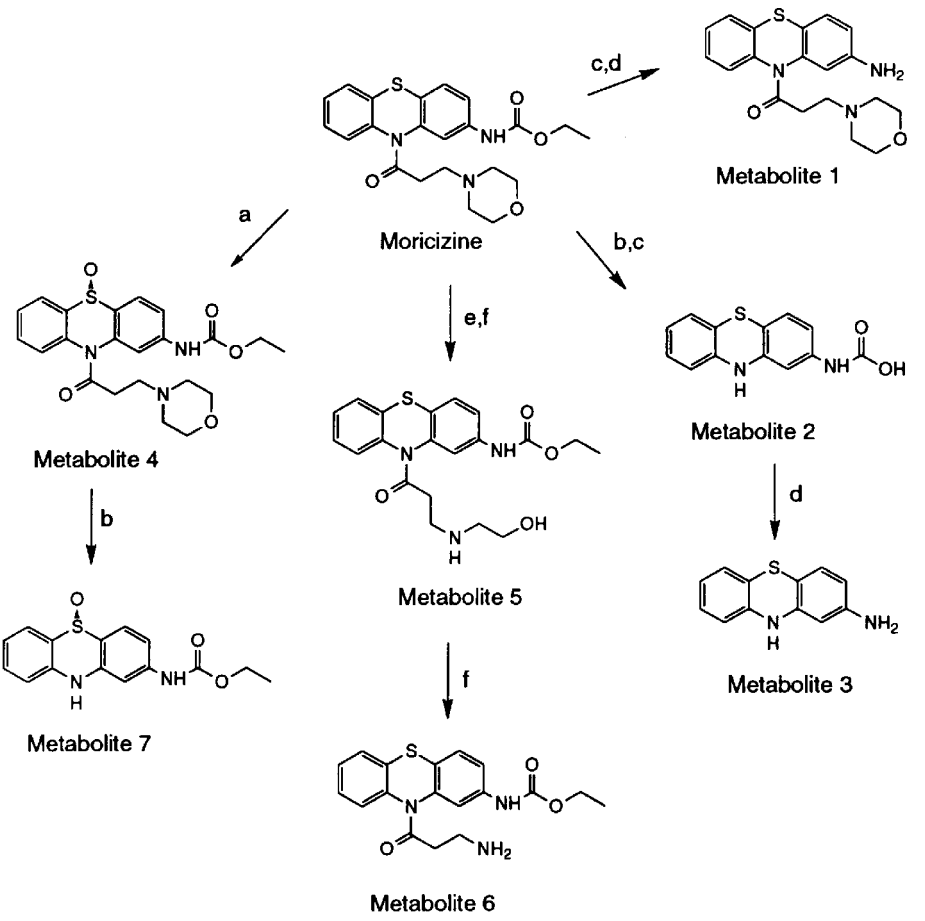


Figure 8. Proposed pathways of moricizine biotransformation in man. (a) Sulphoxidation; (b) hydrolysis of the amide bond of the heterocyclic nitrogen atom; (c) hydrolysis of the ester bond; (d) decarboxylation of carbamic acid; (e) morpholine ring opening; (f) oxidative *N*-dealkylation.

In the present study, several human moricizine metabolites were characterized that contain either the intact morpholino side chain (metabolite 1) or the alkyl amine in some form (metabolites 5 and 6). This alkyl amine functionality has been proposed to be instrumental in the activity of class 1 antiarrhythmics by enhancing the binding of drug to the sodium channel receptor (Sheldon *et al.* 1991). In addition, the pK_a of the amine functionality has been shown to affect drug binding. Ethacizin, a tertiary amine analogue of moricizine with a pK_a = 9·3, is protonated at physiological pH. The sodium channel receptor off-rate of ethacizin in perfused cardiac Purkinje cells was greatly increased as compared with moricizine, which, with a pK_a = 6·4, exists largely as the free base at physiological pH (Makielski 1990). Ethacizin has been shown to be a more potent antiarrhythmic drug than moricizine (Kaverina *et al.* 1984).

Based on these findings, metabolite 1, (2-amino-10-(3-morpholinopropionyl) phenothiazine), with the intact morpholine, would be expected to exhibit antiarrhythmic activity similar to that of moricizine. Likewise, metabolite 5, (ethyl {10-[*N*-(2′-hydroxyethyl)3-aminopropionyl] phenothiazin-2-yl}carbamate), would be expected to have a pK_a between 7 and 8, and therefore an activity between that of

moricizine and ethacizin, although the hydroxyl moiety, with its ability to hydrogen-bond, could affect binding to the sodium channel in an unforeseen manner. The primary amine functionality of metabolite 6, (ethyl[10- (3-aminopropionyl) phenothiazin-2-yl] carbamate), would have a pK_a between 8 and 9, and would be protonated at physiological pH. Therefore, metabolite 6 would be expected to exhibit potency similar to ethacizin.

In summary, moricizine is converted to multiple metabolites in man, some of which contain the structural requirements for antiarrhythmic activity.

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