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Title: Pyrilamine in the horse: I. Isolation, identification and synthesis of its major urinary metabolite

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SUMMARY

Pyrilamine is an antihistamine used in human and equine medicine. Since antihistamines produce central nervous system effects in horses, pyrilamine has the potential to affect the performance of racing horses. Three metabolites of pyrilamine were isolated from horse urine and identified by GC/MS and NMR analysis. The metabolites were isolated from horse urine only after treatment with β-glucuronidase. These metabolites resulted from *O*-demethylation (O-desmethylpyrilamine), pyridine ring hydroxylation (5'-hydroxypyrilamine), and *O*-demethylation and *N*-demethylation (N,O-desmethylpyrilamine). A TLC screening test was developed for detecting the O-demethylated metabolite in basic extracts of horse urine treated with β-glucuronidase. The metabolite was extracted and treated with BSTFA to produce the trimethylsilyl (TMS) ether derivative for GC/MS analysis. O-Desmethylpyrilamine was synthesized and characterized by GC/MS, NMR and IR. The mass and retention time of the TMS derivative of the synthesized compound were compared to those of isolated metabolite treated in the same manner.

INTRODUCTION

Pyrilamine (N-[(4-methoxyphenyl)methyl]-N',N'-dimethyl-N-2-pyridinyl-1,2-ethanediamine, Figure 1) is an antihistamine that is an H_1-receptor antagonist used extensively in human and veterinary medicine for symptomatic relief of allergic reactions. Although the behavioral effects of pyrilamine on the horse are unknown, general side-effects of antihistamines include sedation or central nervous system stimulation, depending on the medication, dose, and route of administration (Douglas, 1985). Pyrilamine is classified by the Association of Racing Commissioners International as a class 3 agent, and its use in racing horses is thereby prohibited because it may alter the performance of horses during competition. Therefore, methods for detection and identification of antihistamines and/or their metabolites in biological samples from horses is needed for effective control of these agents. Detection of pyrilamine and/or its metabolites in post-race blood or urine samples may lead to significant sanctions against trainers.

Detection and identification of pyrilamine in urine samples has been hampered because the drug is extensively metabolized, and only small amounts of the parent drug are excreted in urine (reference). The objective of this study was to identify and synthesize a major equine metabolite of pyrilamine

MATERIALS AND METHODS

Horses:

Six healthy mares weighing between 450 and 500 kg were used in this study. The horses ranged in age from 3 to12 years and were either Thoroughbreds or Standardbreds. The animals were maintained on grass hay and feed (12% protein), which was a 50:50 mixture of oats and an alfalfa-based protein pellet. Horses were fed twice a day. The animals were vaccinated annually for tetanus and were dewormed quarterly with ivermectin (MSD Agvet, Rahway, NJ). A routine clinical examination was performed before each experiment to assure that the animals were healthy and sound. The horses were placed in box stalls and feed was withheld for two hours before and eight hours after drug administration. Water was available *ad libitum*.

Pyrilamine administration:

For the metabolite identification studies, a balloon-tipped catheter was placed in the bladder of each horse for urine sample collection. Pyrilamine, as pyrilamine hydrochloride aqueous solution (20 mg/ml, Histosol®, Norden Laboratories), was administered by deep intramuscular injection into the gluteal muscles at a total dose of 600 mg/horse (1.20 to 1.33 mg/kg body weight). Urine samples were collected before and from 0 to l, 1 to 2, 2 to 3, 3 to 4, 4 to 6, 6 to 8. and at 24 hours after drug administration. Urine samples were immediately frozen and stored at -5°C until laboratory analysis.

Reagents:

Pyrilamine hydrochloride injectable solution (Histosol®) was purchased from Norden Laboratories (Lincoln, Nebraska); D-saccharic acid 1,4-lactone monohydrate and deuterated dichloromethane (CD_2Cl_2, 99.98% isotopic purity) were purchased from Aldrich Chemical Co. (Milwaukee. WI). Analytical TLC plates (silica gel 60 F_{254}, 5 x 10 cm,. 0.25 mm thickness) were purchased from EM Science, Inc. (Cherry Hill, NJ) . Preparative TLC plates (silica gel 60 F_{254}, 20 x 20 cm, 0.5 mm thickness) were purchased from Analtech (Newark, DE). Dragendorff reagent solution was prepared by combining 10 ml of bismuth subnitrate:glacial acetic acid:water (2:25:100, w/v/v), 10 ml of 40% aqueous potassium iodide, 20 ml of glacial acetic acid, and 100 ml of water. Folin-Denis reagent was prepared by refluxing 10 g of sodium tungstate, 2 g of molybdosilicic acid, 5 ml of concentrated phosphoric acid, and 50 ml of water for 2 hours; the cooled mixture was diluted to 100 ml with water. β-Glucuronidase type L-II (from *Patella vulgata*) was obtained from Sigma Chemicals, St. Louis, MO. N,O-bis(trimethylsilyl)-trifluoroacetimide with 1% trimethylchlorosilane (BSTFA with 1% TMCS) was obtained from Pierce, Rockford, IL.

Thin layer chromatographic (TLC) analysis of urine:

Urine samples were extracted under the following conditions and the resulting isolates were subjected to analytical TLC (Detra ,*et al*, 1985).

Extraction of urine:

A 9ml urine aliquot from each collection interval was alkalinized with 2 ml of 1.0 M sodium carbonate solution containing bromothymol blue (200 mg/liter). The mixtures were then extracted with 5 ml of dichloromethane:isopropanol (3:1) by end-over-end rotation at 20 rpm for 5 min. The phases were separated by centrifugation at 1000 x g for 5 min and the aqueous phases were discarded. The organic phases were transferred to clean 5ml conical centrifuge tubes and evaporated under a flow of nitrogen in a water bath at 40 to 45°C.

Glucuronidase hydrolysis and extraction of urine samples:

Two 5ml urine aliquots from each collection interval were each acidified with 2 ml of 1.0 M sodium acetate buffer (pH 4.5) and 1 ml of β-glucuronidase reagent (type L-II from *Patella vulgata*, 5,000 units/ml in water) was added. The contents of each tube were vortex-mixed and then heated at 65°C for 3 hours.

The mixtures were cooled to room temperature and 0.5 ml of 10% ascorbic acid in water was added to each tube. The pH of each mixture was adjusted. to 8.5-9.2 with 6 N hydrochloric acid or concentrated ammonium hydroxide:water (1:1) as needed. The mixtures were extracted with 5 ml of dichloromethane:isopropanol (10:1) by end-over-end rotation at 20 rpm for 5 min and centrifuged at 1000 x g for 5 min. The aqueous phases were discarded and the organic phases were transferred to clean tubes. The organic phases were then extracted with 3 ml of 1 N sulfuric acid, the organic phases were discarded, and the aqueous phases were transferred to clean tubes.

After addition of 0.2 ml of 10% ascorbic acid to each tube, the pH was adjusted to 8.5-9.2

with concentrated ammonium hydroxide solution. Then, 5 ml of dichloromethane:isopropanol (10:1) was added to each tube and extracted as above.

Verification of glucuronic acid conjugation by glucuronidase and sulfatase inhibition:

The β-glucuronidase reagent, type L-II from *Patella vulgata*, is labeled as also containing sulfatase activity (29,000 sulfatase units per 1500000 β-glucuronidase units). The presence of glucuronic acid conjugates of pyrilamine metabolites was demonstrated by inhibiting β-glucuronidase activity by the addition of the competitive inhibitor D-saccharic acid 1,4-lactone monohydrate and by inhibiting sulfatase activity by substitution of phosphate buffer for acetate buffer in separate experiments.

Three 5ml aliquots of the 0 to 1 hour urine samples were acidified by the addition of 2 ml of 1 M sodium acetate buffer (pH 5.0) and mixed with 1 ml of β-glucuronidase/sulfatase reagent (5000 units/ml). Then 0.1 ml of water was added to the first aliquot, 0.1 ml of 8.1 mM D-saccharic acid 1,4-lactone monohydrate to the second aliquot, and 0.1 ml of 8.1 mM D-saccharic acid 1,4-lactone monohydrate to the third aliquot. The mixtures were incubated at 40°C for 3 hours and extracted as described above.

Sulfatase activity in the β-glucuronidase/sulfatase reagent was inhibited by substituting 0.4 M potassium phosphate buffer for 1 M sodium acetate buffer. A 5ml aliquot of the 0 to 1 hour urine sample was acidified with 2 ml of 0.4 potassium phosphate buffer (pH 5.0) and mixed with 1 ml of β-glucuronidase/sulfatase reagent (5000 units/ml). A 5ml aliquot of the 0 to 1 hour urine sample was acidified with 1 M sodium acetate buffer and processed with the test sample to serve as a control. These mixtures were incubated at 40°C for 3 hours and then extracted as described above.

Thin Layer Chromatographic Analysis of Urine Extracts:

Residues obtained from urine extracts were analyzed by TLC using Merck precoated silica gel 60 F-254 plates (0.25 mm thickness). Each residue was dissolved in 20 µl of ethyl acetate and 1 µl was spotted on the origin of each of 7 TLC plates. Each TLC plate was developed a distance of 5.0 cm in one of the solvent developing systems described in Table 1.

The locations of pyrilamine metabolites were visualized by their blue fluorescence under 350nm UV light, absorption under 254nm UV light, and color formation after sequential application of Folin-Dennis spray reagent followed by exposure to ammonia fumes and Dragendorff spray reagent followed by 5% sodium nitrite solution..

Isolation of metabolites for structural analysis:

Preparative isolation of pyrilamine metabolites extracted from a composite of the 2 to 6 hour urine samples was performed using the β-glucuronidase hydrolysis method described above. The hydrolysis was carried out on 300 ml of urine. The organic extract was evaporated in a 500ml boiling flask at 45°C in a flash evaporator. The residue was dissolved in 500 µl of methanol-ethyl acetate (1:1) and subjected to preparative TLC on 20 cm x 20 cm silica gel GF preparative TLC plates (0.5 mm thickness, Analtech). The extract was streaked along the origin, and the TLC plate was developed 10 cm in solvent system A (Table 1). Metabolite zones were visualized under 350nm and 254nm UV light and were separately collected with Kontes 3ml zone

collectors. The metabolites were eluted from the silica gel with 3-4 ml of methanol-ethyl acetate (1:l). The methanol eluates were concentrated under nitrogen on a water bath at 45°C.

Each partially purified metabolite was rechromatographed in solvent system 1, after which the plate was dried in air and then developed in solvent system B (Table 1). The zones were located and collected as before.

Gas chromatography/mass spectroscopy (GC/MS) analysis of isolated urine metabolites:. Trimethylsilylation of metabolites for qualitative GC/MS analysis :

The isolated metabolites were mixed separately with 20 µl BSTFA in capped, conical tubes and heated for 30 minutes at 65°C. The mixtures were diluted with 20 µl of ethyl acetate before GC/MS analysis (Detra, *et al*, 1985).

Low resolution GC/MS analysis of metabolites isolated from urine extracts:

The GC/MS instrument was a Hewlett-Packard model 5970 mass selective detector and model 5890 gas chromatograph with splitless injector, capillary direct interface, and autosampler. Aliquots (2 µl) of the BSTFA-treated sample extracts were injected via the splitless injector at 280°C on to a 15 m x 0.251 mm i.d. DB-l capillary column (0.25 µm film thickness, J & W Scientific, Rancho Cordova, CA) at an initial column oven temperature of 150°C. After 1 min, the temperature of the column oven was increased at a rate of 20°C/min to a final temperature of 280°C and was maintained at that temperature for 12.5 min. Helium was used as the carrier gas at a flow rate of 1 ml/min. The mass spectrometer was operated under electron-impact ionization conditions at 70 eV. Perfluorotributylamine was used as the reference compound.

High resolution GC/MS analysis:

High resolution GC/MS analysis of isolated metabolites was done at the Ohio State University Physical Chemistry Instrument Center using a VG model 70-250S double focusing magnetic sector instrument and a Hewlett-Packard model 5890 gas chromatograph. Aliquots of the BSTFA-treated sample extracts were injected via the splitless injector at 280°C onto a 30 m x 0.251 mm i.d. capillary column (0.25 µm film thickness, DB-1. J & W Scientific) at an initial column oven temperature of 100°C, After 3.0 min, the column oven temperature was increased at a rate of 15°C/min to a final temperature of 280°C. Helium was used as the carrier gas at a flow rate of 1 ml/min. All spectra were obtained under electron-impact ionization conditions at 70 eV and a source temperature of at 200°C. Perfluorokerosene was used as the reference compound.

Nuclear magnetic resonance (NMR) spectrometry of isolated metabolites:

The purified metabolites were dissolved in 1-2 ml of HPLC-grade ethyl acetate and transferred to 5 x 250 mm NMR tubes (Wilmad, Buena, NJ). The ethyl acetate was concentrated to dryness under nitrogen at room temperature (20°C), and the tubes were then placed in a vacuum desiccator containing phosphorus pentoxide for 2 d before ^{1}H NMR analysis. The residues in the NMR tubes were dissolved in CD_2Cl_2 for ^{1}H NMR analysis with a Bruker AM-500 system.

Synthesis and characterization of O-desmethylpyrilamine:

Chemical synthesis of O-desmethylpyrilamine (ODMP) was accomplished by debenzylation of pyrilamine, followed by alkylation, reduction, diazotization and hydrolysis (Figure 2). Debenzylation of pyrilamine (4) was performed in trifluoroacetic acid, which gave the highest yield of the debenzylated amine (6). Alkylation of the debenzylated amine with p-nitrobenzyl bromide in the presence of sodium amide in toluol provided a moderate yield (28 %) of the nitro compound (7), which was reduced with tin in concentrated hydrochloric acid to produced the amine (8). After diazotization with sodium nitrite in sulfuric acid and following acidic hydrolysis at 70 °C, the desired ODMP (1) was obtained. The crude product was purified by column chromatography on silica gel. Fine white crystals were obtained after crystallization from ethyl ether.

The synthesized product was characterized by NMR, GC/MS, Fourier transform infra-red (FT-IR) spectrometry, and melting point determination. ^{1}H-NMR (200 MHz) and ^{13}C-NMR (50 MHz) spectra were recorded on a Varian Gemini AC-200 with tetramethylsilane as internal standard. GC/MS analysis was performed on a Hewlett-Packard Model 6890 gas chromatograph equipped with a Model 5972A mass selective detector. IR spectra were recorded on Perkin-Elmer 1640 FT-IR spectrometer. Melting points were uncorrected.

RESULTS

Identification of metabolites

Using qualitative GC/MS analysis, ^{1}H NMR analysis and TLC, three metabolites of pyrilamine were identified in basic extracts of urine treated with β-glucuronidase to hydrolyze glucuronic acid conjugates. The metabolites were identified as N-(4-hydroxybenzyl)-N',N'-dimethyl-N-2-pyridinyl-1,2-ethanediamine, designated as ODMP (1), N-(4-methoxybenzyl)-N',N'-dimethyl-N-2-(5-hydroxypyridinyl)-1,2-ethanediamine, designated as 5'-hydroxypyrilamine (2), and N-(4-hydroxybenzyl)-N'-methyl-N-2-pyridinyl-1,2-ethanediamine, designated as N,O-desmethylpyrilamine (3) (Figure 1).

Pyrilamine metabolites, ODMP (1), 5'-hydroxypyrilamine (2), and N,O-desmethyl-pyrilamine (3), were isolated from urine samples subjected to hydrolysis with β-glucuronidase (*Patella vulgata* preparation) and extracted under alkaline conditions (Table 1). Metabolite 2 exhibited intense blue fluorescence when illuminated under 254nm or 350nm UV light and all metabolites reacted with Folin-Denis and Dragendorff spray reagents suggesting the presence of an aromatic hydroxyl group and a basic nitrogen atom in each. Pyrilamine metabolites 1, 2 and 3 were not detected in extracts of alkaline urine unless the urine was first treated with β-glucuronidase.

Mass spectral analysis (Figure 3) of the trimethylsilyl (TMS) derivative of metabolite 1 under electron-impact ionization conditions indicated an apparent molecular ion of m/z 343 and a base peak ion of m/z 179, both of which provided valuable diagnostic evidence for the proposed structure of ODMP (1). First, the increased molecular mass (58 amu) of the base peak ion of the metabolite relative to that of pyrilamine was consistent with the loss of a methyl group and addition of one TMS group. Secondly, in an analysis of the fragmentation pattern of pyrilamine, we proposed that the base peak ion at m/z 121 represented the 4-methoxybenzyl group.

Consequently, the base peak-ion at m/z 179 displayed by the metabolite indicated that the 4-methoxybenzyl group in the metabolite had undergone O-demethylation and TMS formation.

The mass spectrum (Figure 4) of the TMS derivative of metabolite 2 exhibited an apparent molecular ion at m/z 373, no significant ion at m/z 214, and a base peak ion at m/z 121. The net increase of 88 amu in the molecular ion relative to that of pyrilamine suggested the addition of oxygen and one TMS group. The ion at m/z 121 indicated that the 4-methoxybenzyl group was not altered, and the lack of an ion at m/z 214 indicated the pyridine ring was not altered.

The NMR spectrum (Figure 5) of metabolite 2 showed only two pyridine ring protons, a singlet at 7.93 ppm and a doublet at 6.51 ppm, However, the apparent doublet at 7.15 ppm represented three protons, indicating that a third pyridine ring proton was hidden by one pair of benzylic protons. Due to the downfield location of the peak at 7.93 ppm, it was attributed to the proton on C-6 and its singlet nature indicated it was on the carbon atom between the nitrogen atom and the substituted position. The peak at 6.51 ppm was attributed to the proton at C-3 because of its similarity to the proton at 6.46 ppm in pyrilamine. Decoupling the protons at 7.15 ppm converted the peak for the proton at C-3 into a singlet, indicating that the hidden proton was at C-4, On this basis, the tentative structure of the second metabolite was assigned as 5'-hydroxypyrilamine.

GC/MS and NMR analysis indicated the identity of the minor metabolite 3 to be N-(4-hydroxybenzyl)-N'-methyl-N-2-pyridinyl-1,2-ethanediamine, designated as N,O-desmethylpyrilamine (data not shown). The observation of the abundance of ODMP relative to the other two metabolites in post-administration equine urine indicated that detection of pyrilamine administration would focus on ODMP (data not shown)..

Characterization of synthesized ODMP

The total ion chromatogram of the TMS derivative of chemically synthesized ODMP was generated by GC/MS (Figure 6a). The electron impact (EI)-mass spectrum of the predominate 12.6 min peak seen in the total ion chromatogram indicated a major abundance response at m/z 179 (Figure 6b). Table 2 lists the interpretation of the EI-mass spectrum peaks indicating the molecular ion at m/z 343. This interpretation is consistent with the identification of the synthesis product as ODMP.

NMR characterization data for the synthesized *4-[[[2-(dimethylamino)ethyl]-2-pyridinylamino]methyl]-phenol* (ODMP) was as follows: ^{1}H-nmr (200 MHz, $CDCl_3$): δ (ppm) 2.32 (s, 6 H), 2.58 (t, 2 H, *J* 7.3 Hz), 3.64 (t, 2 H, *J* 7.4 Hz), 4.59 (s, 2 H), 6.39-6.56 (m, 2 H), 6.60 and 6.94 (2 ′ $_{H}$ AB, 2 ′ 2 H, *J* 8.4 Hz), 7.36 (dt, 1 H, *J* 7.7 Hz, *J* 1.8 Hz), 8.12 (dd, 1 H, *J* 1.8 Hz), 8.8-9.3 (bs, 1 H); ^{13}C-nmr (50 MHz, $CDCl_3$): δ (ppm) 45.26 (high 64), 45.57 (16), 51.02 (16), 55.92 (23), 105.89 (24), 111.81 (27), 115.71 (50), 128.16 (77), 128.66 (11), 137.37 (26), 147.80 (35), 156.11 (14), 157.99 (12). FT-IR (KBr) resulted in absorption maxima at 1595, 1501, 1436, 1247 cm^{-1}. The melting point range for synthesized ODMP was119-120 °C.

DISCUSSION

Pyrilamine metabolites, N-(4-hydroxybenzyl)-N',N'-dimethyl-N-2-pyridinyl-1,2-ethanediamine (ODMP, 1), N-(4-methoxybenzyl)-N',N'-dimethyl-N-2-(5-hydroxypyridinyl)-1,2-ethanediamine, (5'-hydroxypyrilamine, 2), and N-(4-hydroxybenzyl)-N'-methyl-N-2-pyridinyl-1,2-ethanediamine, (N,O-desmethylpyrilamine, 3) (Figure 1) were detected by TLC in extracts of glucuronidase-treated urine indicating that the metabolites were excreted as glucuronic acid conjugates (the preparation of β-glucuronidase from *Patella vulgata* contains sulfatase activity that is inhibited by 0.1 M phosphate buffer). These metabolites were not detected by TLC analysis when the activity of β-glucuronidase was competitively inhibited by the addition of D-saccharic acid 1,4-lactone. Furthermore, the yields of the metabolites, based on semiquantitative TLC analysis, were not diminished by inhibition of sulfatase activity by substitution of phosphate buffer for acetate buffer during β-glucuronidase/sulfatase treatment. These findings indicated that the metabolites were eliminated in urine as glucuronic acid conjugates. The excretion of ODMP-O-glucuronide was later confirmed by MS/MS analysis.

Tentative structural assignment based on mass spectral and NMR data collected on ODMP isolated from urine was confirmed by the unambiguous synthesis of the metabolite. The efforts to obtain ODMP from pyrilamine was initially unsuccessful. Inducing the demethylation of pyrilamine in different conditions (i.e. trimethylsilyl iodide, pyridine chloride, acids, LiCl/collidine) failed to produce the desired phenol. In most cases, the debenzylated amine (6) was obtained (Figure 2). Consequently, it was decided that this material would be used for the synthesis of the ODMP metabolite.

Qualitative detection of pyrilamine administration to horses was initially accomplished by TLC analysis of extracts of 5ml aliquots of urine samples collected from 0-1 through 24 hours after intramuscular doses of 600 mg per horse (1.20 to 1.33 mg/kg of body weight). Since this dose is the recommended therapeutic dose for relief of allergic reactions in horses, this method would be suitable for detecting the administration of pyrilamine at doses that might affect performance of the horse.

Confirmation of pyrilamine administration to horses was accomplished by GC/MS identification of ODMP . The presence of ODMP, the most abundant metabolite based on semiquantitative TLC analysis, was confirmed as its TMS ether derivative by electron-impact ionization GC/MS of the basic extract of β-glucuronidase (*Patella vulgata*) treated urine samples collected through 24 hours after intramuscular drug administration.

ACKNOWLEDGMENTS

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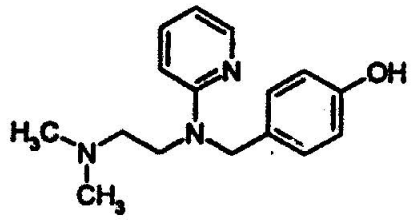
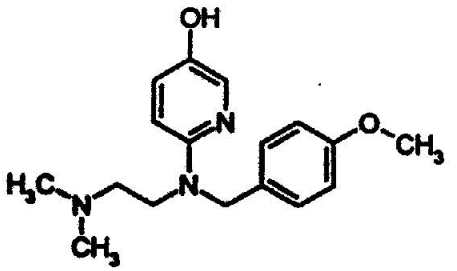
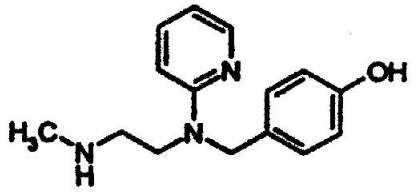


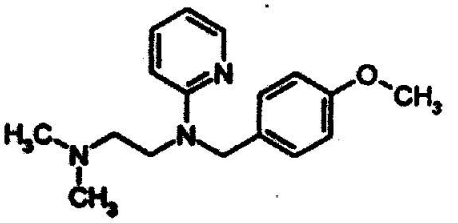
(1) O-desmethylpyrilamine



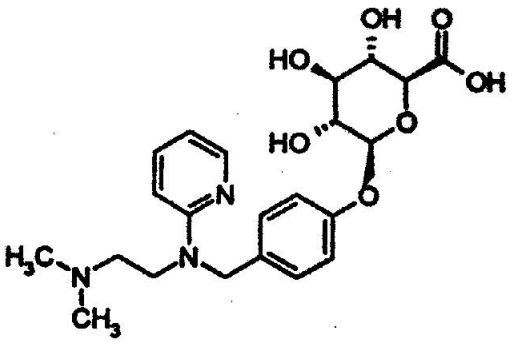
(2) 5'-hydroxypyrilamine



(3) N,O-didesmethylpyrilamine



(4) pyrilamine



(5) O-desmethylpyrilamine glucuronide

Figure 1

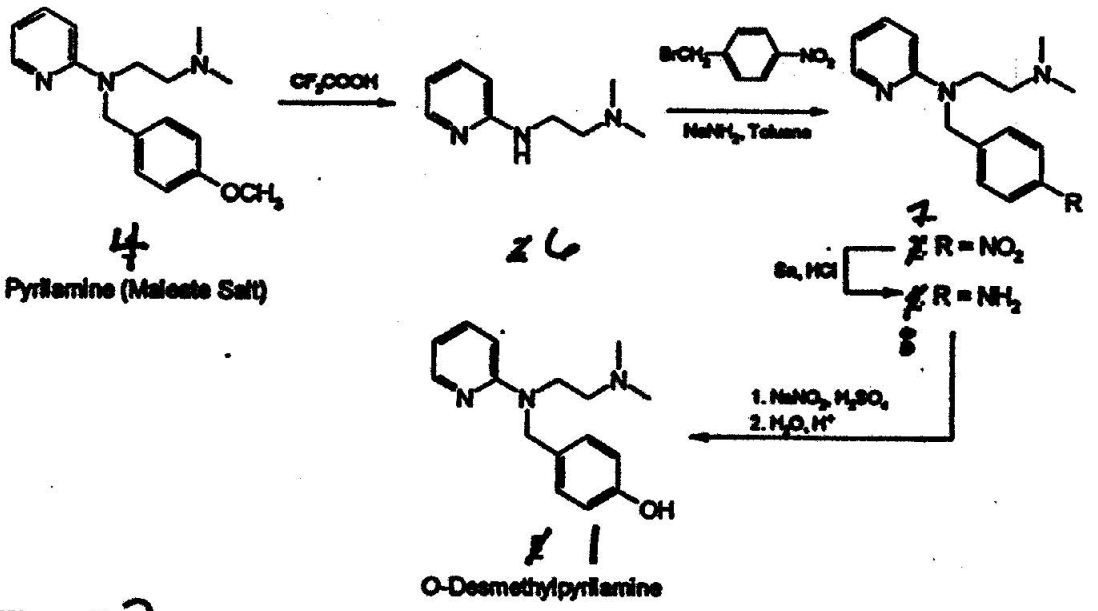


Figure 2

(note change in Compound #'s)

3

Figure 4 (S2ms)

MS Analysis
metabolite 1 -TMS

Figure 4B (55mc)

MS

Metabolite 2- TMS

Table 1 Thin layer chromatographic R_f values for pyrilamine, O-desmethylpyrilamine, 5'-hydroxypyrilamine, and N,O-desmethylpyrilamine on Merck precoated silica gel 60 F-254 TLC plates (0.25 mm thickness)

	Solvent System	
Compound	A	B
Pyrilamine	0.75	0.40
O-desmethylpyrilamine	0.59	0.10
5'-hydroxypyrilamine	0.43	0.09
N,O-desmethylpyrilamine	0.29	0.20

A: ethyl acetate:methanol:ammonium hydroxide solution (80:15:5, v/v)
B: chloroform:methanol:propionic acid (72:18:10, v/v)

Table 2 Interpretation of the principal ions seen in Figure 4b, in order of decreasing m/z value

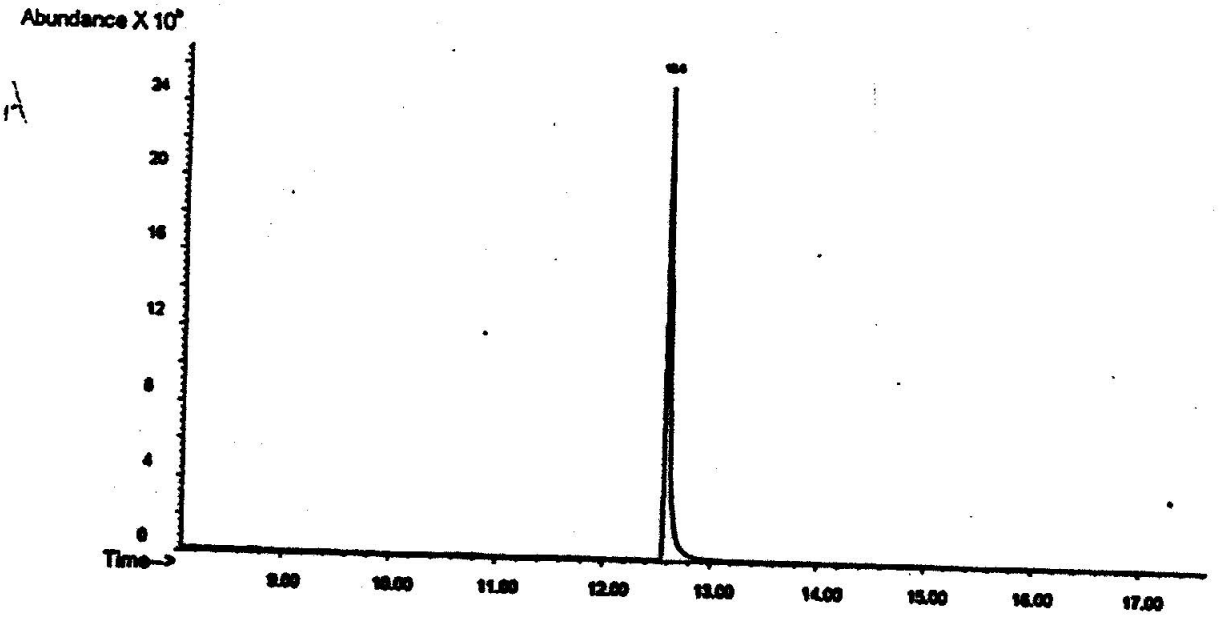
Peak, m/z	Interpretation
343	Molecular ion, M+
328	M+ minus methyl
285	M+ minus $CH_2N(CH_3)_2$
272 cluster	pyridyl-NCH_2O-TMS
179	CH_3-N-(pyridyl ring)-$CH_2CH_2N(CH_3)_2$
163 cluster	CH_3-N-(pyridyl) ring)-$CH_2CH_2NCH_3$
149	N-(pyridyl ring)-$CH_2CH_2NCH_3$
135	CH_3-N-(pyridyl ring)-$CH=CH_2$
119	N-(pyridyl ring)-$CH=CH_2$
106	CH_3-N-(pyridyl ring)
89	O-TMS
73	TMS
72	$(CH_2)_2N(CH_3)_2$
58	$CH_2N(CH_3)_2$

5

Figure 第4 (5mg)

NMR spectrum

Metabolite 2



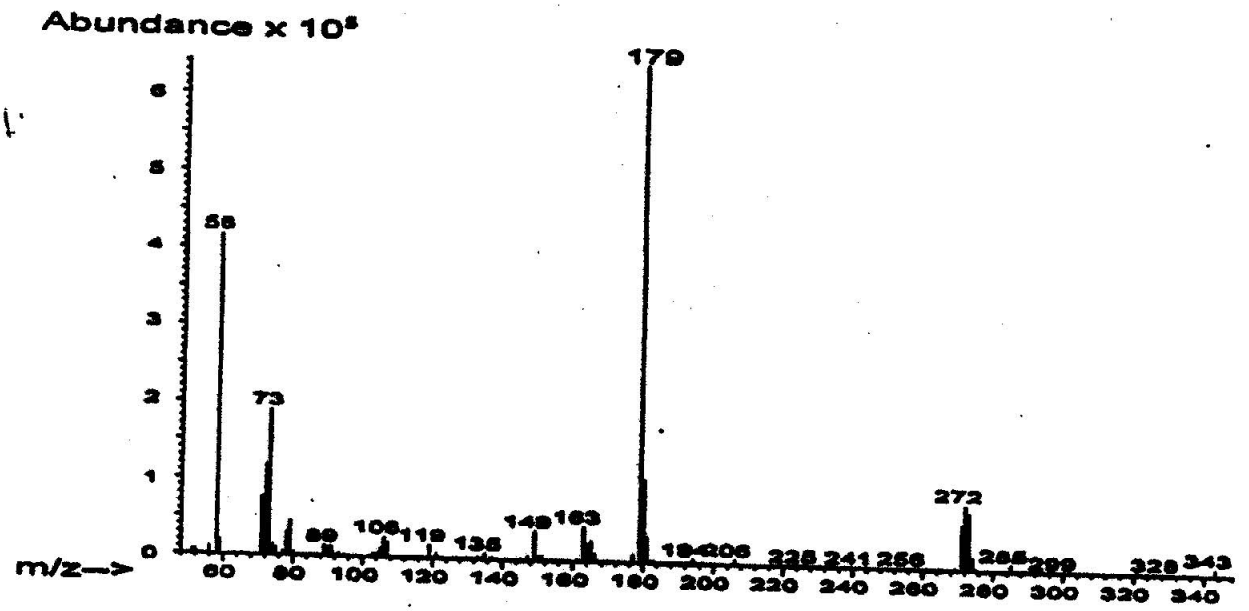


Figure 6 a) Total ion chromatogram of the synthesized O-desmethylpyrilamine compound; b) EI-mass spectrum of the 12.6 min peak seen in Figure 6a.