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Synthesis of a deuterium labeled variant of the rat hepatocarcinogen, methapyrilene

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SUMMARY

Methapyrilene, I, once a commonly found antihistaminic drug, has been synthesized with four deuterium labels, three of which are on the thiophene ring. Initially, thiophene was deuterated by solvolysis of the tetramercuric acetate-derivative with deuterium chloride. Carbonylation of the thiophene ring at the 2-position, by the addition of dimethylformamide to the 2-lithio-derivative, gave [3,4,5-d_3]thiophene-2-carboxaldehyde. Condensation of this aldehyde with 2-aminopyridine was followed by reduction of the resulting imine with sodium borodeuteride to yield the N-([3,4,5-d_3]-2-thienyl[d]-methylene)-2-aminopyridine, VIII. Alkylation of the lithium salt of VIII with dimethylaminoethylchloride gave N,N-dimethyl-N'-[2-pyridyl]-N'-([3,4,5-d_3]-2-thienyl-[d]methylene)-1,2-ethane diamine, I, in good yield. Combustion analysis, 500 mHz NMR, and mass spectrometry confirmed the identity of the product which will be useful for metabolic studies.

KEY WORDS: Methapyrilene, deuterium labeled antihistamines

INTRODUCTION

Methapyrilene (N,N-dimethyl-N'-[2-pyridyl]-n'-(2-thienylmethylene)-1,2-ethanediamine) was first synthesized about 40 years ago (1), and subsequently patented as an antihistaminic drug (2). After years of usage the drug was found to be a potent rat hepatocarcinogen (3,4), though not carcinogenic in either guinea pigs or hamsters (5) and subsequently removed from the marketplace. Some closely related structural analogues have been reported as non-carcinogenic in rats (6,7).

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Little metabolic work with methapyrilene existed until an abstract appeared stating that at least 4 polar compounds appeared in rat urine after dosing with I along with small amounts of I (8). A subsequent in vitro rabbit liver metabolic study with I found several metabolites, including the desmethyl- and N-oxide compounds (9). We have examined the metabolism of I in

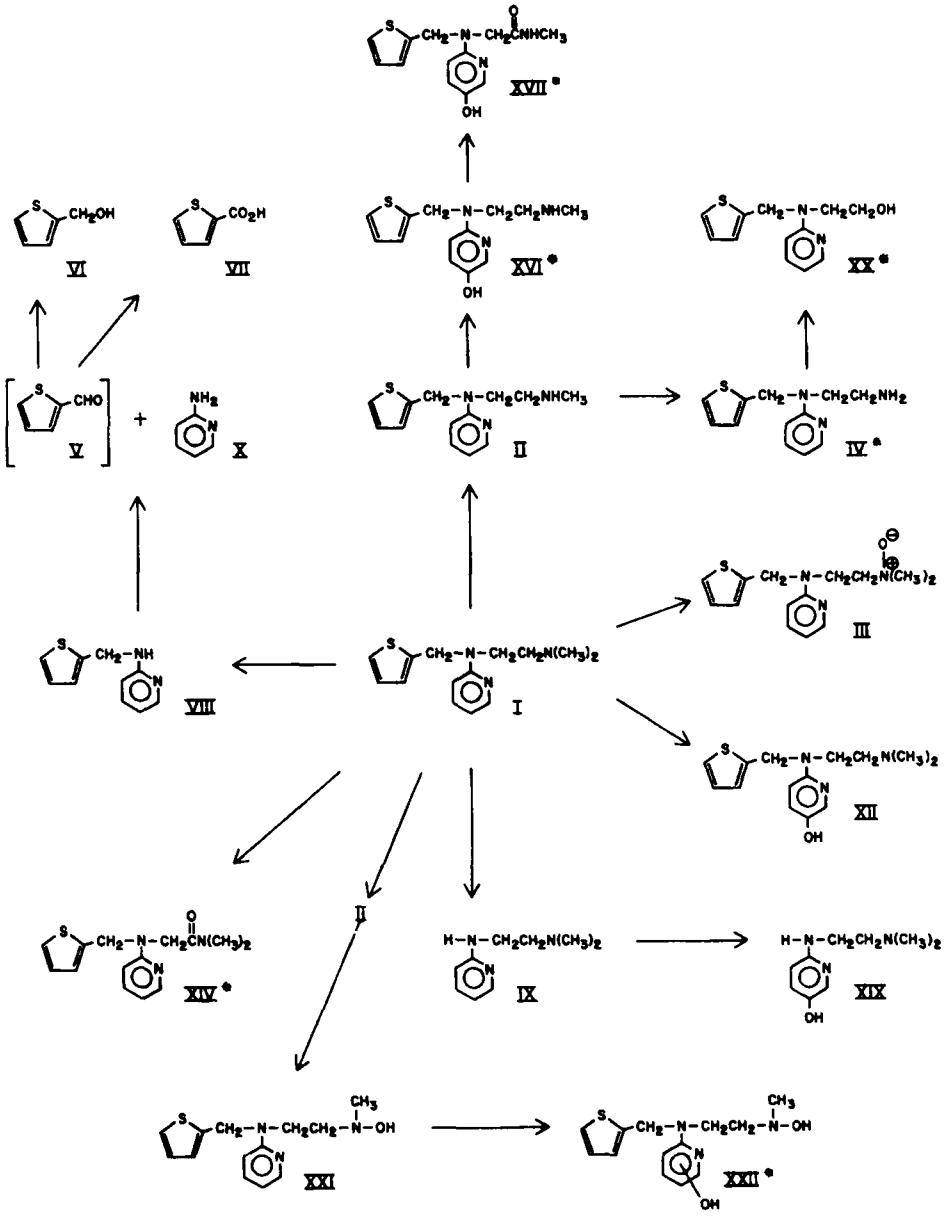


Fig. 1: Proposed metabolic-disposition pathways for methapyrilene, I, based upon what is known to date.

the rat, both in vitro (10), in vivo (11) and in other species (12,13). We have also reported that chronic pretreatment with I changes the metabolism of I in the rat in a variety of ways (14), and that some of the metabolites of I are mutagenic after nitrosation (15). A summary of the known metabolic work is depicted in Figure 1. In all of these studies, however, there are unknown metabolites formed and a portion of the original dose of I (Fig 1) is unaccounted for. We decided, therefore, that it was necessary to synthesize a stable isotope labeled variant of I in order to further elucidate its metabolism via gas-chromatography-mass spectrometry. This report describes the first synthesis of a deuterium labeled variant of I (Fig 1). Metabolic results may require that still other stable isotope variants will be needed.

MATERIALS AND ANALYTICAL METHODS

Mercuric acetate, acetic acid, dimethylformamide, cumene, sodium carbonate, ethyl acetate, diethyl ether, hexane and other common reagents were all purchased from Fisher Scientific, Tustin, California, in the highest purity available. Thiophene, deuterium chloride, deuterium oxide, sodium borodeuteride, n-butyllithium in hexane, dimethylaminoethylchloride, thiophene-2-carboxaldehyde, and 2-aminopyridine were all obtained from Aldrich Chemical Co., Milwaukee, Wisconsin in the best available grade.

Melting points were taken on a Thomas Hoover melting point apparatus and are uncorrected. Combustion analyses were performed by Galbraith Laboratory, Knoxville, Tennessee. Analytical thin layer chromatography plates were obtained from E. Merck AG, Darmstadt, Germany.

Gas-chromatography - Mass spectrometry

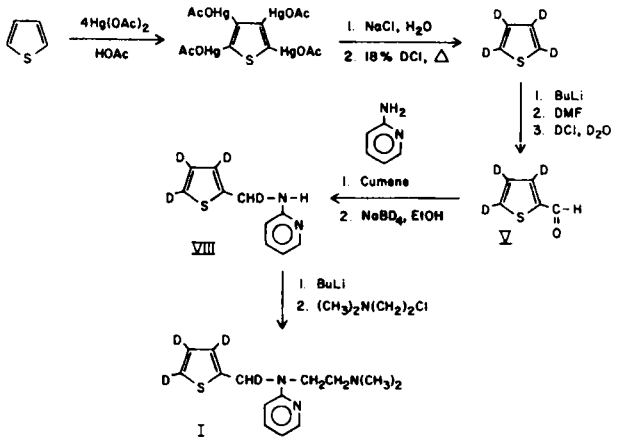
Both synthetic intermediates and the final product were analyzed by GC-MS on a Hewlett Packard 5987A GC-MS system operating in the electron impact mode (70 e.v.) equipped with a 12 M fused silica OV-1 capillary column (0.2 mm i.d. and 0.33 μm film thickness) and operating as a splitless capillary-direct instrument. The injector, transfer line, analyzer, and source temperatures were set at 250°C, 310°C, 200°C and 200°C, respectively. Ultra pure helium was utilized as the carrier gas.

Synthesis

The general outline of the chemical synthesis utilized is depicted in Scheme I. N,N-Dimethyl-N'-[2-pyridyl]-N'-([3,4,5-d_3]-2-thienyl[d]methylene)-1,2-ethanediamine, I, was obtained in 5 steps starting from commonly available reagents (thiophene and 2-aminopyridine). Individual steps are described below.

Thiophene-d_4

This substance was prepared basically as reported earlier (16). One hundred sixty eight grams of 98% mercuric acetate (0.53 mol) were stirred into



Scheme I: Flow chart of the synthesis of deuterium-labeled methapyrilene, I.

500 ml glacial acetic acid in a 2 l Erlenmeyer flask and 10.5 g (0.125 mol) pure thiophene added. The mixture was heated on a steam bath for 4 hr, which became very thick with a white precipitate. The mixture was diluted with 500 ml water, filtered, and washed 1× with 300 ml water. This tetramecurial acetate of thiophene was then added to 1 l water containing 100 g sodium chloride and allowed to stand in a hot water bath for 3 hr. The solution was then cooled, filtered, and washed with 200 ml water. The resulting fine white precipitate was air-dried overnight, and then dried under a 0.01 mm Hg pressure for 36 hr. The fine white powder then weighed 120 g.

The powder was then stirred with 37% DCl (50 ml) and 75 ml D_2O at reflux for 4 hr. A condensor and trap was then connected to the flask and distillation carried out until all efflux of thiophene had ceased; about 10 ml (including D_2O, with the thiophene on top). Separation and drying over molecular sieves gave 4.0 g (40%). GC-MS: m/z 88 (100%; thiophene-d_4, parent ion) m/z, 60 (60%). Retention time (45°C; 1.0 min).

[3,4,5-d_3]thiophene-2-carboxaldehyde, V(17)

Five-tenths of a gram thiophene-D_4 (5.7 mmol) was dissolved in 30 ml dry ether under N_2. Six-tenths of a milliliter of 10.4 M n-butyllithium (6.2 mmol) was then added dropwise at room temperature and stirred for 3 hr. Six-tenths of a milliliter (0.58 g or 8.0 mmol) of neat dimethylformamide were then added and allowed to stir overnight. The reaction was then adjusted to pH 7 with DCL/D_2O (37%) and 10 ml D_2O added. The reaction was extracted with ether (2×), dried over sodium sulfate, and rotary evaporated to a yellow oil. This was distilled at 90°C/30 mm Hg, to give a clear liquid, (76% yield). GC-MS: m/z 115 (80%, parent ion); m/z 114 (100%); m/z 86, (15%); m/z 58, (15%). Retention time (45°C; 1.10 min.)

N-([3,4,5-d_3]-2-thienyl[d]methylene)-2-aminopyridine, VIII

Two and four-tenths grams (trideuterothiophene)-2-carboxaldehyde (20 mmol) was added to 2.82 g 2-aminopyridine (30 mmol) in 150 ml cumene and refluxed overnight, with subsequent azeotropic distillation of the water formed. Thin-layer chromatography on silica gel plates showed only 2 spots: a reaction product and excess 2-aminopyridine. The entire crude reaction mixture was then added to a stirred solution of 1.0 g (23.6 mmol) of sodium borodeuteride in 150 ml isopropyl alcohol, and stirred overnight. The isopropyl alcohol was removed, organic layer was diluted with ether and extracted with 50 ml H_2O. The organic layer was then dried over sodium carbonate and evaporated in vacuo to give 4.7 g of an orange solid. The solid was dissolved in ethyl acetate and flash chromatographed over silica gel with 50:50 ethylacetate/hexane eluent. The oil eluting was crystallized from ethyl acetate/hexane to give 2.5 g of yellow crystals. A second crop weighed 0.7 g

for a total yield of 82%. Recrystallization from hexane gave plates, mp 78°C. NMR (500 mHz, $CDCl_3$): δ 1.80 (bs, 1H, NH); δ 4.67-4.70 (m, 1H, HCD-thienyl); δ 6.42 (d, 8.5 hz, 1H, H-3); δ 6.60-6.62 (m, 1H, H-5); δ 7.40-7.43 (m, 1H, H-4); δ 8.12 (dd, 4.5 hz, 1 hz, 1H, H-6). Anal. calculated for: $C_{10}H_6D_4N_2S$; C, 61.83; H, 5.19; N, 14.42; found: C, 61.87; H, 5.34; N, 14.32. GC-MS retention time (90°C, 5.0 min, then 10°/min to 270°C; 12.07 min). The mass spectrum is shown in Fig. 2 along with the mass spectrum of unlabeled VIII for comparison.

N,N-dimethyl-N'-[2-pyridyl]-N'-([3,4,5-d_3]-2-thienyl[d]methylene)-1,2-ethanediamine, I

The final product, deuterium labeled methapyrilene, was synthesized by a variation of the original synthesis of some structurally related compounds (18). One gram (5.2 mmol) of VIII was dissolved in 70 ml dry toluene. One-half of a milliliter of 10.4 m, n-butyllithium in hexane was then added at

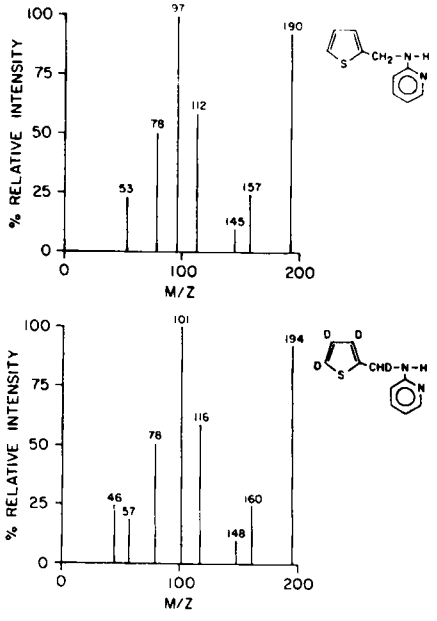


Fig. 2: Mass spectra of both unlabeled and deuterium-labeled N-(2-thienylmethylene)-2-aminopyridine, VIII.

room temperature and stirred for 15 min. A solution of dimethylamino-ethylchloride (1.0 g; 7.0 mmol; prepared by basifying the hydrochloride salt and extracting with 50 ml toluene and drying over molecular sieves) was added, and the solution refluxed overnight. Quenching with ammonium hydroxide, was followed by extraction with ethylacetate, evaporation, and chromatography on alumina (neutral, activity 3). Elution with 1:3 ethyl acetate/hexane gave a yellow oil which was dissolved in methanolic HCl, and removal of the methanol gave a yellow solid which was recrystallized from isopropyl alcohol/ethyl acetate twice to give white crystals, 0.55 g (40%); m.p. 159–160°C. NMR (500 mHz, $CDCl_3$): δ 2.84 (s, 6H, $(CH_3)_2$)N); δ 3.25 (t, 7.5 Hz, 2H, CH_2); δ 4.13 (t, 7.3 Hz, 2H, CH_2); δ 4.86 (s, 1H, CHD); δ 6.64–6.67 (m, H, H-5); δ 6.74 (d, 8.5 Hz, 1H, H-3); δ 7.48–7.50 (m, 1H, H-4); δ 8.15 (dd, 4Hz, 1.5Hz, 1H, H-6).

Anal. calc'd for: $C_{14}H_6D_4N_2SCl$; C, 55.71; H, 6.68; N; 13.92; found: C, 55.60; H, 6.88; N 13.71. GC-MS retention time (90°C, 5.0 min, then 10°/min to 270°C; 15.16 min). The mass spectrum is shown in Figure 3, along with the mass spectrum of unlabeled I for comparison.

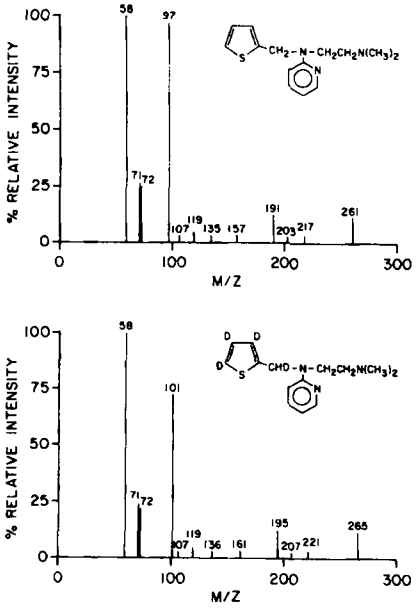


Fig. 3: Mass spectra of both unlabeled and deuterium-labeled methapyrilene, I.

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