solution is diluted to 0.6 mL with Mops buffer and 100 μL is removed and diluted 20-fold for noninhibited control assays. A 5-μL aliquot of 1.0×10^{-2} M DFP in ethanol is added to the remaining 0.5 mL of enzyme solution and incubated for 3 min, during which period inhibition of the enzyme is complete. After the incubation period, the entire solution is added to a 1 × 20 cm glass column packed to a bed height of 12 cm with Sephadex G-50, 50–100 mesh, the solution is eluted under suction with Mops buffer, and 1.0- to 2.0-mL fractions are collected. The enzyme elutes primarily in the 2- to 4-mL fraction, and DFP primarily in the 7- to 9-mL fraction. The elution of DFP in the AChE fraction was negligible as evidenced by incubating noninhibited AChE with the 2- to 4-mL fraction and assaying for enzymatic activity. For reactivation studies, candidate reactivators are dissolved to 1.0×10^{-2} M in water and diluted to 3.0×10^{-6} to 2.0×10^{-3} M in 100 μL of inhibited enzyme solution plus the quantity of buffer required to give a 200 μL final volume. These solutions are incubated at 25 ± 0.2 °C in a shaker bath, and 25-μL aliquots are removed at timed intervals. The 25-μL aliquots are added to 965 μL of Mops buffer plus 10 μL of 1.0×10^{-2} M pNPA, and the enzyme activity is assayed. Thus, the final concentrations in the assay solutions are as follows: 1×10^{-9} M inhibited AChE, 3.00×10^{-6} to 2.00×10^{-5} M reactivator, and 1.0×10^{-3} M substrate. Observed enzyme activities are corrected (see Results) for spontaneous and reactivator-catalyzed hydrolysis of substrate to give net activities: 100% enzyme activity is defined as the activity restored by incubation of inhibited enzyme with 1.0×10^{-3} M **3** for 60 min at 25 °C.

Ellman Procedure. The general procedure described above is followed except that lower enzyme concentrations are required. Typically, a 10-μL aliquot of stock AChE is diluted to 0.6 mL in Mops buffer and incubated with a 4-μL aliquot of 1.0×10^{-2} M DFP for 10 min. From the 1- to 4-mL column fraction of inhibited enzyme, 400-μL aliquots are withdrawn and diluted to 600 μL with Mops buffer and the volume of reactivator stock solution required to give a 3.00×10^{-6} to 2.00×10^{-3} M reactivator concentration. At timed intervals, 20-μL aliquots of the enzyme plus reactivator solution are withdrawn and assayed as described above. Thus, the final concentrations in the assay solution are as follows: 1.0×10^{-10} M AChE, 3.00×10^{-6} to 2.00×10^{-5} M reactivator, 3.0×10^{-4} M DTNB, and 7.5×10^{-4} M acetylthiocholine.

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Trapping of Metabolically Generated Electrophilic Species with Cyanide Ion: Metabolism of Methapyrilene

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The popular antihistamine methapyrilene [*N*,*N*-dimethyl-*N*′-(2-pyridyl)-*N*′-(2-thienylmethyl)-1,2-ethanediamine] recently has been shown to be a potent hepatocarcinogen. Metabolic studies with rabbit liver 100000*g* microsomal preparations have resulted in the partial characterization of the in vitro metabolic profile of methapyrilene. Evidence for the formation of the *N*-oxide and the three possible carbinolamines resulting from the NADPH-dependent oxidation of the (dimethylamino)ethyl side chain nitrogen and carbon atoms of methapyrilene is presented. Attempts to trap iminium ion intermediates with electrophilic alkylating potential by coincubating methapyrilene with sodium cyanide have led to the isolation of *N*-(cyanomethyl)normethapyrilene. The possibility of characterizing the iminium ion intermediate that would result from the oxidative deamination of the dimethylamino moiety was precluded by the chemical instability of the corresponding α-cyano amine, which undergoes a spontaneous retro-Michael reaction and hydrolysis to the corresponding amide. The results are discussed in terms of the metabolic activation of methapyrilene to potential alkylating species.

Recent studies have established that the widely used histamine H_1-receptor antagonist methapyrilene [*N*,*N*-dimethyl-*N*′-(2-pyridyl)-*N*′-(2-thienylmethyl)-1,2-ethanediamine (1)] is a potent hepatocarcinogen.[1] Although the carcinogenic properties of many xenobiotics are thought to be mediated by metabolically generated electrophilic intermediates that form covalent bonds with nucleophilic functionalities present on biomacromolecules,[2] it is not clear what bioactivation pathway(s) methapyrilene might undergo. The in vivo conversion of methapyrilene to *N*-nitroso metabolites, at one time considered a possible metabolic activation pathway for this compound,[3] does not appear to account for its carcinogenicity.[4]

We have been interested in the possible metabolic conversion of tertiary amines to electrophilic iminium ions. These reactive species, which theoretically may form by ionization of intermediary α-carbinolamine metabolites,[5] have been demonstrated indirectly by isolation of the corresponding α-cyano amines from liver microsomal coincubations of nicotine and 1-benzylpyrrolidine with the nucleophilic trapping agent sodium cyanide.[6] In the present paper we report the results of our studies on the in vitro metabolism of methapyrilene and of our attempts to characterize the metabolic conversion of this hepatocarcinogen to reactive iminium ions.

Results and Discussion

In Vitro Metabolism of Methapyrilene in the Absence of Sodium Cyanide. Our metabolic work employed NADPH-supplemented 100000*g* microsomal preparations obtained from phenobarbital-induced rats or from un-

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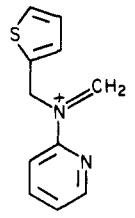
treated, 6-month-old, male Dutch rabbits. The qualitative metabolic patterns were essentially identical with these two systems. Therefore, the results summarized in this paper are those obtained with the rabbit, since metabolite production was greater with rabbit liver microsomes.

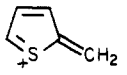
In order to estimate the extent to which methapyrilene is metabolized by this system, a quantitative GC assay of the parent drug was developed in which pyrilamine [1-[*N*-(2-pyridyl)-*N*-(4-methoxyphenyl)amino]-2-(dimethylamino)ethane] served as internal standard. Over a 1-h period, a 1 mM solution of methapyrilene was metabolized to the extent of 33 to 50%. In the absence of NADPH or an NADPH-generating system, methapyrilene was recovered quantitatively.

GC analysis of the basic fraction isolated from postincubates showed the presence of several peaks in addition to starting drug. These peaks appeared to be metabolites of methapyrilene, since they were not present in extracts obtained from incubations which had not been supplemented with NADPH. A very rough estimate of the amounts of metabolites formed indicated that the volatile base fraction (pH 10 methylene chloride extract) accounted for less than 50% of the methapyrilene consumed in the microsomal incubations. Attempts were made to isolate additional metabolites by methylene chloride extraction of the incubation mixtures at pH values ranging from 4 to 8. GC analysis of the extracts both before and after treatment with diazomethane, however, did not reveal additional metabolites.

Methapyrilene metabolites were characterized initially by GC–EIMS analysis. The EI mass spectrum of methapyrilene displayed a parent ion [M^+ 261 (8)] and fragment ions [*m/e* 203 (7), 97 (77), and 78 (36) corresponding to structures i, ii, and iii, respectively]. The fragment ions



i (*m/e* 203)



ii (*m/e* 97)

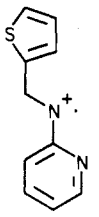
iii (*m/e* 78)

ii and iii appeared in the EI mass spectra of all the methapyrilene metabolites characterized in this study. Therefore, these metabolites must have resulted from alterations of the *N*,*N*-dimethylaminoethyl moiety of the parent drug.

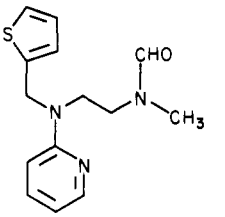
The quantitatively most important metabolite (up to 45% of the methapyrilene metabolized) detected in the GC–FID tracing of the base fraction proved to be the N-demethylated product **2**. The GC–EI mass spectrum displayed the parent ion [M^+ 247 (30)] and the expected fragment ions [i (100) and ii (19)]. An attempt to synthesize this compound (see below) led to a product which displayed a GC–EI mass spectrum identical with that of this metabolite and an NMR spectrum consistent with the proposed structure. Efforts to obtain an analytically pure form of this product, however, failed.

Four additional metabolically derived products were detected in the GC tracing of the postincubate extract. According to the GC–FID response, these products in toto represent less than 4% of the methapyrilene metabolized in this microsomal system. The structure of one minor metabolite has been shown to be 2-[(2-thienylmethyl)amino]pyridine (**3**) by GC–EIMS analysis [M^+ 190 (69), *m/e* 97 (ii, 100) and 78 (iii, 56)] and by comparison with an authentic sample.

The remaining three products have been tentatively assigned structures **6**, **7**, and **9**, primarily on the basis of GC–EIMS data. The EI mass spectrum of the compound assigned the *N*-formyl structure **6** displayed strong parent [M^+ 218 (36)] and fragment [ii (100) and iii (94)] ions. Additionally, as expected, a strong fragment ion, iv, cor-



iv (*m/e* 189)



10

responding to the loss of CHO, appeared at *m/e* 189 (75). On the basis of information obtained from our synthetic work described below, compound **6** is most likely a spontaneous breakdown product of the aminoacetaldehyde derivative **5**. Compound **5** is the metabolic product expected to result from oxidative deamination of methapyrilene via fragmentation of the intermediate carbinolamine **4**. A second minor metabolite characterized by GC–EIMS which is consistent with the formation of the carbinolamine **4** is the amide derivative **7**. The EI mass spectrum of this material displayed a parent ion [M^+ 275 (16)] and fragment ions [i (14), ii (100), and iii (10)]. The absence of fragment ions at masses 246 or 247, corresponding to the loss of CHO or CO,[7] argues against the isomeric *N*-formyl derivative **10**.

The final minor product characterized from the metabolism of methapyrilene is the *N*-oxide **8**. As expected, this metabolite was not detected as such because of its thermal instability.[8] The thermal decomposition product of **8** observed in the GC–EIMS of the postincubate extract has been assigned the vinylamine structure **9** [M^+ 216 (6), *m/e* 97 (ii, 100) and 78 (iii, 33)]. We prepared the *N*-oxide **8** by oxidation of methapyrilene with H_2O_2 in order to examine its behavior under GC–EIMS conditions. Although the *N*-oxide could not be obtained in analytically pure form, the spectral characteristics of the product were consistent with the desired product (see Experimental Section). GC–EIMS analysis of synthetic **8** confirmed that it undergoes thermal decomposition to the vinylamine **9**, as well as to methapyrilene and the two secondary amines **2** and **3**. Since metabolic formation of **9** would be unprecedented and since metabolic N-oxidation of tertiary amines is quite common,[9] we have assumed that compound **9** is an artifact resulting from the thermal decomposition of the metabolically generated *N*-oxide **8**. The ratio of thermal decomposition products obtained with synthetic **8** (**2**/**3**/**9** = 1:8:8) differs from the corresponding ratio observed in the GC tracing of the postincubate extract (**2**/**3**/**9** = 50:3:2). Although thermal breakdown of the *N*-oxide also might account for the presence of the [(thienylmethyl)amino]pyridine metabolite (compound **3**), the high metabolic yield of normethapyrilene (**2**) compared to that generated thermally from the *N*-oxide confirms that compound **2** is a true metabolite of methapyrilene.

In Vitro Metabolism of Methapyrilene in the Presence of Sodium Cyanide. We next turned our attention to the cyanide trapping studies. The metabolic profile summarized in Scheme I suggests three reaction

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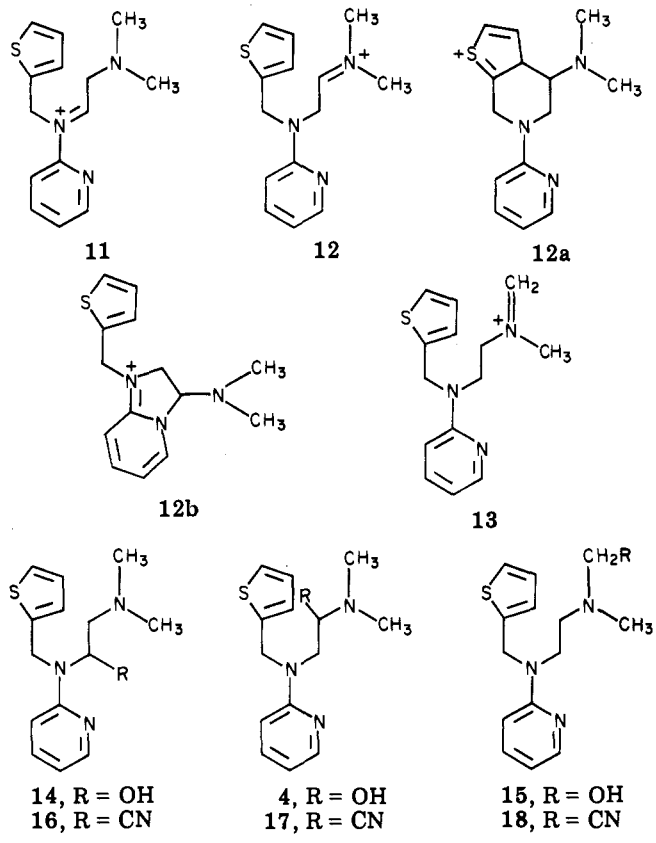
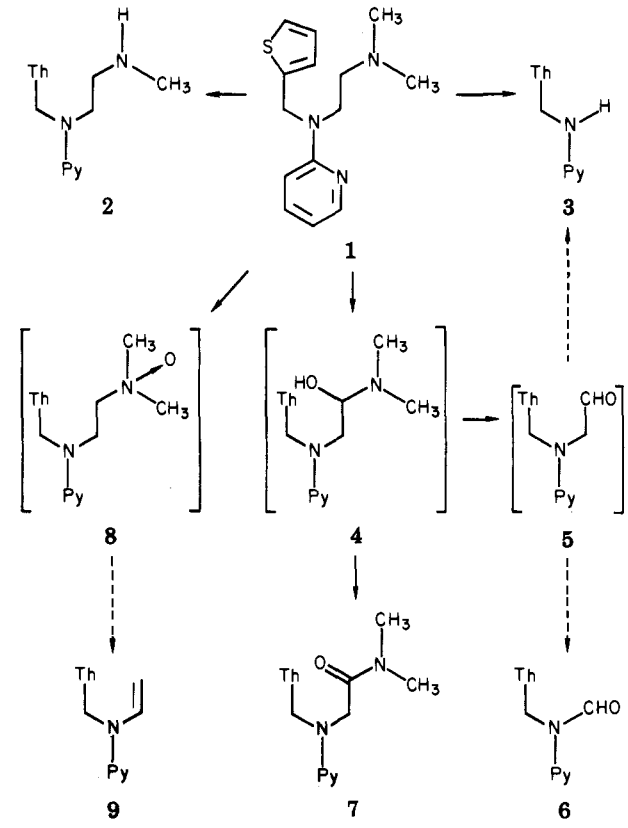
pathways for methapyrilene that could involve the production of iminium ions **11–13**. The corresponding carbinolamines are represented by structures **14**, **4**, and **15** and the corresponding α-cyano amines that would be formed upon cyanide attack of these iminium ions by structures **16**, **17**, and **18**, respectively. It is noteworthy that the stability of iminium ion **12** could be enhanced through delocalization of the positive charge as depicted in structures **12a** and **12b**.



The incorporation of cyanide ion into these and other possible metabolically generated electrophilic intermediates was monitored with the aid of ^{14}C-labeled sodium cyanide (0.25 mCi/mmol). Preliminary studies indicated that an NADPH-supplemented microsomal incubation of a solution 1 mM in methapyrilene and 1 mM in ^{14}C-labeled sodium cyanide led to appreciable (up to 12%, average 6%) incorporation of the label into material extractable into methylene chloride at pH 10. Furthermore, since the extractable activity from incubation mixtures not supplemented with NADPH was essentially at background levels (equivalent to 0.05% incorporation), we concluded that the formation of organic cyano products was a metabolically dependent process.

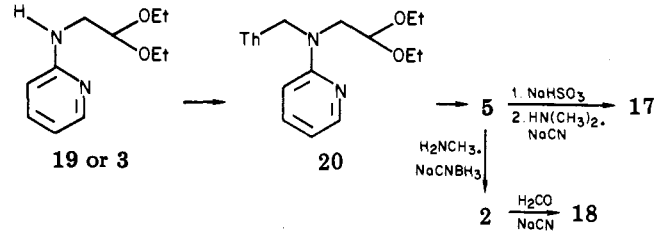
In order to carry out product analysis, 64 postincubation mixtures were pooled and the base fraction was worked up. A preparative TLC was run and radioscanning of the plate indicated a principal radioactive zone, which after partitioning into aqueous acid and back-extraction at pH 10 proved to be homogeneous (UV absorption and ^{14}C content) by reverse-phase HPLC. Both probe CIMS (MH^+ 287) and GC–EIMS (M^+ 286) confirmed that the structure of this product corresponded to α-cyano amines **16**, **17**, or **18**. The presence of an ion at mass 203 (14%) corresponding to fragment ion i eliminated structure **16** as a candidate for this cyano adduct. The presence of additional fragment ions, including a weakly intense response at *m/e* 259 due to loss of HCN, was consistent with both compounds **17** and **18**. In order to assign the structure of

Scheme I. Metabolic Profile of Methapyrilene (1) Based on Metabolites Characterized from Extracts of Rabbit Liver 100000*g* Microsomal Postincubates[a]



[a] Structures in brackets are proposed, but not detected, intermediates. Dashed arrows indicate conversions that are not likely to be enzyme catalyzed but rather to occur spontaneously or thermally during GC–EIMS analysis. Th represents the 2-thienyl moiety and Py the 2-pyridyl moiety of methapyrilene.

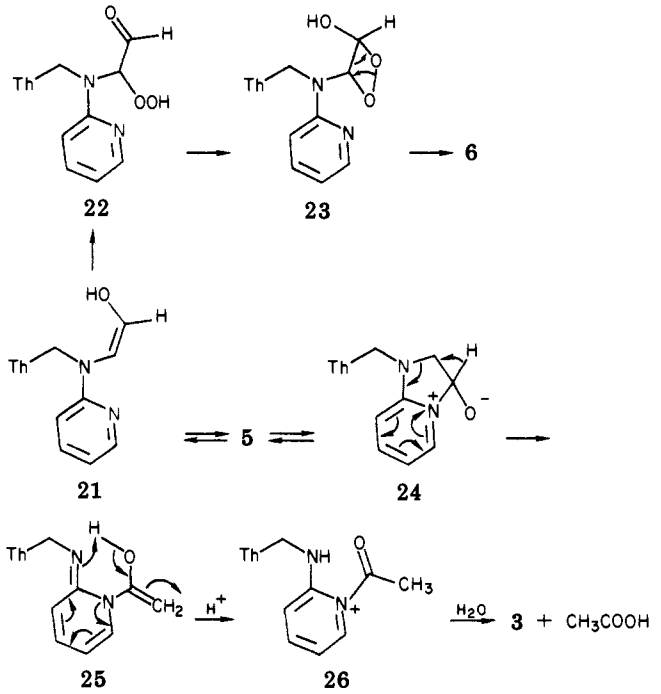
Scheme II. Synthetic Pathways Leading to Normethapyrilene 2 and α-Cyano Amines 17 and 18



the metabolically generated cyano adduct unambiguously, the synthesis of both **17** and **18** was undertaken.

The preparation of these compounds [together with normethapyrilene (**2**)] was approached via the aminoacetaldehyde derivative **5** (Scheme II). Both the secondary amine **3** and the amino acetal **19** could be converted to acetal **20**. The alkylation of these 2-aminopyridine derivatives required forcing conditions, probably due to the low nucleophilicity of the nitrogen atom. Hydrolysis of the acetal moiety yielded the desired aldehyde **5**, which proved to be quite unstable but could be characterized by NMR and low- and high-resolution mass spectrometry. This conversion required careful experimental conditions, including the exclusion of atmospheric oxygen and the addition of traces of hydroquinone as a radical scavenger. When exposed to air, compound **5** decomposed to a minor extent to the *N*-formyl derivative **6** and to a major extent, even when stored under nitrogen, to the secondary amine

Scheme III. Proposed Pathways for the Decomposition of Aminoacetaldehyde 5 to the Secondary Amine 3 and the *N*-Formyl Derivative 6[a]



[a] Th refers to the 2-thienyl moiety.

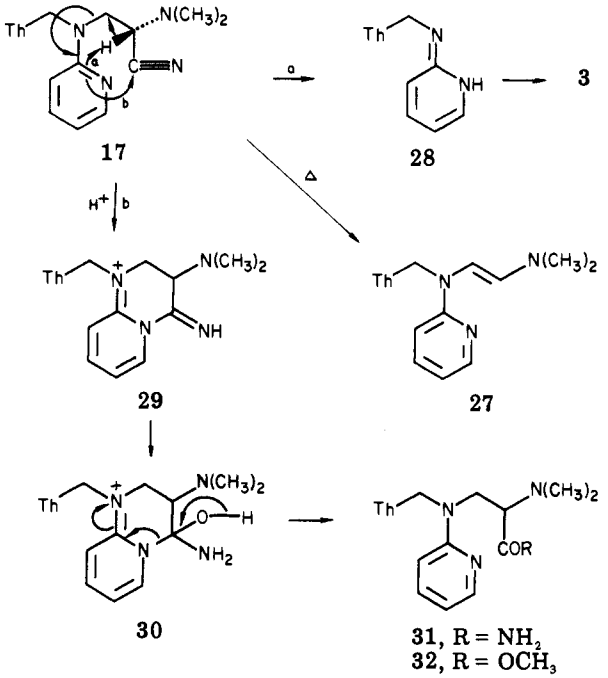
3. The presence of compound 6 in decomposed reaction mixtrues was documented by GC–EIMS [M^+ 218 (36) and *m/e* 189 (2v, 40)] and that of compound 3 by GC–EIMS with the aid of an authentic sample.

Possible mechanisms to account for these conversions are depicted in Scheme III. Attack of the enol 21 by dioxygen may lead to hydroperoxide 22 which, through the intermediacy of the oxetane 23, would loose formic acid to give rise to the *N*-formyl derivative 6. Alternatively, aldehyde 5, as its mesomeric zwitterionic isomer 24, may rearrange via enol 25 to the *N*-acetyl intermediate 26, which upon spontaneous hydrolysis would yield secondary amine 3 and acetic acid. NMR analysis of the decomposed reaction mixture clearly displayed a sharp singlet at δ 2.15 characteristic for the methyl group of acetic acid.

Attempted synthesis of α-cyano amine 17 by condensation of aldehyde 5 with dimethylamine in the presence of sodium cyanide failed. The overall conversion could be achieved by allowing the aldehyde 5 to react first with sodium hydrogen sulfite, followed by treatment with dimethylamine and sodium cyanide. Column chromatography provided the desired product as an oil, which was characterized by NMR and mass spectrometry. The GC–EI mass spectrum of the metabolically generated cyano adduct was similar to this product but differed in that compound 17 displayed no parent ion at mass 286 and no fragment ion at mass 190 but did show a moderately intense signal at mass 259. Thermal loss of HCN from 17 would be expected to lead to the enamine 27 which has a nominal mass of 259. High-resolution mass spectral analysis of this ion is consistent with structure 27. A pseudomolecular ion (MH^+) corresponding to protonated 17 was observed at mass 287 in the CI mass spectrum of 17.

Attempts to recover 17 from microsomal preparations or from pH 7.4 aqueous buffer were unsuccessful. Two decomposition products, the secondary amine 3 and the amide 31, have been characterized spectroscopically from an aqueous solution of 17. Scheme IV depicts likely

Scheme IV. Chemical Decomposition Pathways of α-Aminonitrile 17[a]



[a] Th refers to the 2-thienyl moiety.

pathways to account for the observed chemical behavior of 17. Intramolecular proton abstraction promotes the retro-Michael reaction to form 28, which tautomerizes to 3. Hydrolysis of the cyano group is probably facilitated by formation of intermediate 29, which upon addition of water forms the cyclic tautomer, 30, of the final product, amide 31. When treated with methanolic HCl, 17 again underwent a variety of reactions. As expected, a principal product detected in the GC–EI mass spectrum of this reaction mixture was the α-amino ester 32 [M^+ (319, 1), *m/e* 260 (1), 203 (50), 116 (25), 97 (100), 78 (20)].

A retrospective analysis of the mass spectral data obtained from the methapyrilene–sodium cyanide metabolic studies did not reveal the presence of amide 31 in postincubate extracts. On the other hand, since the instability of 17 precludes its detection in these incubations, the question concerning the metabolic formation of iminium ion 12 remains unresolved.

The synthesis of the *N*-(cyanomethyl) derivative 18 proceeded via normethapyrilene (2). Condensation of the crude aldehyde 5 with methylamine in the presence of sodium cyanoborohydride led to 2, which was obtained as an oil and characterized by NMR and GC–EIMS. The GC–EIMS behavior of this synthetic material was identical with the principal metabolite of methapyrilene. Treatment of crude 2 with formaldehyde and sodium cyanide led to 18, which could be purified by preparative TLC and characterized by NMR and mass spectrometry. The GC–EIMS characteristics of synthetic 18 were identical with the radioactive cyano adduct isolated from the methapyrilene–sodium cyanide incubations. In a subsequent incubation study, pure 18 was isolated in an adequate quantity to obtain its NMR spectrum, which proved to be identical with that of synthetic 18.

Summary

The results described above provide evidence that methapyrilene undergoes NADPH-dependent oxidation at all three unique carbon atoms and the nitrogen atom of the (dimethylamino)ethyl side chain. In terms of detectable metabolites, oxidative N-demethylation appears to be the

quantitatively most significant pathway. However, poor mass balance (i.e., percent methapyrilene consumed vs. metabolite yield) and the chemical instability of the aminoacetaldehyde derivative **5** make difficult the assessment of the quantitative significance of the oxidative deamination pathway. Consistent with the high yield of normethapyrilene, cyanide trapping experiments led to the isolation of *N*-(cyanomethyl)normethapyrilene (**18**), which we interpret as evidence for the metabolic conversion of methapyrilene to iminium ion **13**. Whether or not this iminium ion may be involved in the carcinogenic properties of methapyrilene remains unresolved. The experimental evidence presencted in this paper also does not allow us to exclude the possible metabolic formation of iminium ion **12**. Additional approaches to the detection of electrophilic metabolic products of methapyrilene are currently being pursued.

Experimental Section

All reactions were carried out under a nitrogen atmosphere. Solvents for reactions were dried and distilled prior to use. Proton NMR spectra were recorded on a Varian FT-80 (80 MHz) instrument; all spectra were recorded in $CDCl_3$. Chemical shifts are reported in parts per million (ppm) relative to Me_4Si as an internal standard; s = singlet, d = doublet, t = triplet, m = multiplet, br = broad, and Th = 2-thienyl. Infrared (IR) spectra were recorded on a Perkin-Elmer 337 grating IR spectrophotometer. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Analytical gas chromatography (GC) was performed on a Varian Aerograph Series 2100 gas chromatograph with an H_2 flame-ionization detector (FID) utilizing a U-shape 2 m × 2 mm i.d. glass column packed with 3% OV-25 on acid-washed DMCS Chromosorb W (100–120 mesh). Chemical-ionization mass spectra (CIMS) were obtained on an AEI MS-902 instrument modified for chemical ionization. Gas chromatography–electron impact mass spectrometry (GC–EIMS) was performed either on an Infotronics 2400 gas chromatograph interfaced to an AEI MS-12 mass spectrometer or on a 10 m × 0.325 mm i.d. glass capillary column coated with either 0.1% SE-52 or 0.1% OV-1 coupled directly to an Hitachi M-52 mass spectrometer. High-resolution electron-impact mass spectra (HR-EIMS) were obtained on a modified AEI MS-902 instrument. Scintillation counting was performed on a Packard Tri-Carb Model 3375 liquid scintillation spectrophotometer using Aquasol or Aquasol-2 (New England Nuclear) as the scintillation cocktail. Radioscans of the TLC plates were carried out on a Varian Aerograph Radio Scanner Model LB 2722. Analytical (250 μm) and preparative (2000 μm) silica gel TLC plates were purchased from Analtech. Elemental analyses were performed by the Microanalytical Laboratory, University of California, Berkeley.

The synthesis of 2-[(2-thienylmethyl)amino]pyridine [**3**: 45% yield; mp 77–79 °C (lit.[10] mp 78–80 °C); NMR δ 4.72 (s, CH_2), 6.35–7.43 (m, 6 ArH), 8.13 (d, ArH)], *N*-(2-pyridyl)aminoacetaldehyde diethyl acetal[11] [**19**: 47% yield; NMR δ 1.20 (t, 2 CH_3), 3.35–3.84 (m, 3 CH_2), 4.46 (t, CH_2CH), 4.84 (br, NH), 6.34–6.61 (m, 2 ArH), 7.36 (t, ArH), 8.07 (d, ArH)], and 2-(chloromethyl)thiophene[12] [47% yield; bp 78–80 °C (20 mm); NMR δ 4.75 (s, CH_2), 6.85–7.30 (m, 3 ArH)] were achieved according to the cited literature procedures.

***N*-(2-Pyridyl)-*N*-(2-thienylmethyl)aminoacetaldehyde Diethyl Acetal (20). Procedure A.** To a mixture of amine **3** (3.8 g, 20 mmol) and bromoacetaldehyde diethyl acetal (3.94 g, 20 mmol) in 30 mL of anhydrous xylene was added $NaNH_2$ (0.98 g, 25 mmol). The reaction mixture was stirred under reflux for 24 h, after which time additional $NaNH_2$ (0.25 equiv) and bromoacetaldehyde diethyl acetal (0.5 equiv) were added. The same additions were repeated at 48 and 72 h. After 96 h at reflux, the reaction mixture was cooled to room temperature and treated with H_2O (50 mL), and the organic layer was separated. The aqueous layer was extracted twice with CH_2Cl_2, and the combined, dried (K_2CO_3) organic extract was evaporated (in vacuo) to yield a dark-brown oil. The oil was column chromatographed on 50 g of silica gel (40–140 mesh) with CH_2Cl_2 using an ethyl acetate gradient. Distillation of the residue obtained from the desired fractions (2–5% EtOAc/CH_2Cl_2, analyzed by GC) gave a colorless oil in 77% yield: bp 120–125 °C (0.25 mmHg); NMR ($CDCl_3$) δ 1.18 (t, 2 CH_3), 3.40–3.84 (m, 3 CH_2), 4.72 (t, CH_2CH), 4.96 (s, $ThCH_2$), 6.47–7.60 (m, 6 ArH), 8.17 (d, ArH); GC–EIMS 306 (M^+, 18), 261 (9), 203 (9), 189 (6), 103 (89), 97 (100), 78 (35), 75 (78). Anal. ($C_{16}H_{22}N_2O_2$) C, H, N.

Procedure B. To a mixture of the acetal **19** (20 g, 47.6 mmol) and 2-(chloromethyl)thiophene (6.31 g, 47.6 mmol) in 200 mL of anhydrous toluene was added $NaNH_2$ (2.23 g, 51.1 mmol). The reaction mixture was stirred under reflux. Additional $NaNH_2$ (1.1 g, 28.2 mmol) and 2-(chloromethyl)thiophene (3.1 g, 23.4 mmol) were added to the reaction mixture after 45 h and yet again [$NaNH_2$ (0.55 g, 11.7 mmol) and 2-(chloromethyl)thiophene (1.5 g, 11.7 mmol)] after 68 h. After 80 h at reflux, the reaction mixture was cooled, quenched with H_2O (100 mL), and worked up as described in procedure A. A pure (by GC) yellow oil with a strong sulfur odor was obtained in 96% yield after distillation. This oil was repeatedly partitioned into aqueous 2 N HCl and back-extracted at pH 10 into CH_2Cl_2 and was then distilled [bp 135–140 °C (0.15 mm)] to give a clear, odorless oil (pure by GC) in 70% yield. The NMR spectrum of this product was identical with that of the product obtained by procedure A.

***N*-(2-Pyridyl)-*N*-(2-thienylmethyl)aminoacetaldehyde (5).** A few crystals of hydroquinone were added to a solution of acetal **20** (3.7 g, 12 mmol) in 50 mL of 2 N HCl. After heating for 1 h at 80 °C, the now milky reaction mixture was cooled to 0 °C and extracted with 50 mL of CH_2Cl_2. The pH of the aqueous layer was adjusted carefully with Na_2CO_3 to 10. The basic solution was extracted (5 × 50 mL) with CH_2Cl_2, and the combined extract was dried over K_2CO_3 and evaporated to give the desired product as a yellow oil (100% yield): NMR ($CDCl_3$) δ 4.18 (d, CH_2CHO), 4.88 (s, $ThCH_2$) 6.62–7.60 (m, 6 ArH), 8.16 (m, ArH), 9.47 (s, CHO); IR (neat) 1750 (C=O) cm^{-1}; CIMS 233 (MH^+); GC–EIMS 232 (M^+, 7), 203 (10), 189 (23), 97 (100), 78 (24); HR–EIMS Calcd for $C_{12}H_{12}N_2OS$: M^+ 232.0668. Found: M^+ 232.0644. Attempted distillation of this oil gave amine **3** (characterized by GC–EIMS) as the only identifiable product.

1-(Dimethylamino)-1-cyano-2-[*N*-(2-pyridyl)-*N*-(2-thienylmethyl)amino]ethane (17). The crude aldehyde **5** (3.67 g, 12 mmol) was dissolved in a mixture containing 5 mL of H_2O, 10 mL of pH 5.3 acetate buffer, 3 mL of a 42% $NaHSO_3$ solution (1.25 g, 12 mmol), and 35 mL of MeOH. To the stirred reaction mixture warmed to 40 °C was added a 20.15% methanolic solution of dimethylamine (3.2 mL, 14.4 mmol), followed by NaCN (0.59 g, 12 mmol) in 1 mL of H_2O. After 15 min, the reaction mixture was cooled to room temperature and extracted with CH_2Cl_2 (3 × 30 mL). The combined CH_2Cl_2 extract was dried over K_2CO_3, and the solvent was removed in vacuo. The crude oil (3.22 g) was column chromatographed on 360 g of silica gel (40–140 mesh) with CH_2Cl_2 (EtOAc gradient) as the developing solvent. The desired fractions (identified by GC) eluted with 7% EtOAc/CH_2Cl_2 and were combined and evaporated to give the pure (one peak by GC) α-cyano amine as a colorless oil in 9% yield: NMR ($CDCl_3$) δ 2.36 (s, 2 CH_3), 3.71–4.26 (m, NCH_2CHCN), 4.93 (d, $ThCH_2$), 6.55–7.56 (m, 6 ArH), 8.19 (br d, ArH); IR ($CHCl_3$) 2200 (C≡N) cm^{-1}; CIMS 287 (MH^+); GC–EIMS 259 (M^+, 20), 162 (74), 97 (100), 92 (55); HR–EIMS Calcd for $C_{14}H_{17}N_3S$: M^+ 259.1139. Found: M^+ 259.1139.

1-[*N*-(2-Pyridyl)-*N*-(2-thienylmethyl)amino]-2-(methylamino)ethane (2). To a solution of the crude aldehyde **5** (0.57 g, 2.47 mmol) in 7 mL of anhydrous MeOH was added 8.6 mL of a 5.4% methanolic solution of methylamine (14.8 mmol). After the solution was cooled to 0 °C, 2 N methanolic HCl (to adjust the pH to 7) and $NaCNBH_3$ (0.32 g, 5.0 mmol) were added, and the resulting mixture was stirred for 24 h at room temperature. The mixture was then treated with water (15 mL), made basic with solid K_2CO_3, and extracted with CH_2Cl_2 (3 × 25 mL). The combined extract was dried over K_2CO_3 and evaporated to give a brown oil (one peak by GC): NMR ($CDCl_3$) δ 2.42 (s, CH_3), 2.80 (t, CH_2), 3.64 (br t, CH_2), 4.91 (s, $ThCH_2$), 6.47–7.41 (m, 6 ArH),

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8.17 (br s, ArH); GC–EIMS 247 (M^+, 15), 204 (65), 191 (15), 107 (50), 97 (100), 78 (20). Attempts to purify this product by column chromatography were unsuccessful.

1-[*N*-(2-Pyridyl)-*N*-(2-thienylmethyl)amino]-2-[*N*-(cyanomethyl)-*N*-methylamino]ethane (18). To a solution of the crude amine 2 (180 mg, 0.73 mmol) in 3.0 mL of MeOH and 1.0 mL of H_2O was added NaCN (73.5 mg, 1.5 mmol) in 2.0 mL of H_2O, followed by 0.6 mL of a 37.6% aqueous solution of formaldehyde (220 mg, 7.3 mmol). After the solution was stirred for 1 h at room temperature, H_2O (30 mL) was added and the reaction mixture was extracted with CH_2Cl_2 (4 × 25 mL). The combined CH_2Cl_2 extract was dried over K_2CO_3 and evaporated to an oil. The crude oil was then purified by preparative TLC (silica gel) with 1% CH_3CN/CH_2Cl_2 as the developing solvent to yield the product as a yellow oil: NMR ($CDCl_3$) δ 2.39 (s, CH_3), 2.67 (t, CH), 4.86 (s, $ThCH_2$) 6.49–7.42 (m, 6 ArH), 8.14 (br d, ArH); EIMS 286 (M^+, 3), 203 (11), 190 (8), 97 (100), 78 (14); HR–EIMS Calcd for $C_{15}H_{18}N_4S$: M^+ 286.1252. Found: M^+ 286.1276.

1-[*N*-(2-Pyridyl)-*N*-(2-thienylmethyl)amino]-2-(*N*,*N*-dimethyl-*N*-oxoamino)ethane (8). A solution of methapyrilene (2.61 g, 10 mmol) in 20 mL of THF at 0 °C was added to a 10% aqueous solution of H_2O_2 (15 mmol). The solution was allowed to stand at room temperature for 36 h, at which time the solvent was removed by rotary evaporation to yield a viscous oil. The residue in 15 mL of H_2O was made basic with K_2CO_3 and was extracted twice with diethyl ether (to remove starting methapyrilene) and then with CH_2Cl_2 (to recover product). The CH_2Cl_2 extract was dried over K_2CO_3 and evaporated to an oil, which solidified to a white solid upon trituration with benzene and hexane: mp (sealed tube) 69–72 °C; NMR ($CDCl_3$) δ 3.22 (s, 2 CH_3), 3.48 (t, CH_2), 4.20 (t, CH_2), 4.89 (s, $ThCH_2$), 6.60–7.46 (m, 6 ArH), 8.15 (br d, ArH). Anal. ($C_{14}H_{19}N_3OS \cdot H_2O$) H, N; C: calcd, 56.9; found, 57.9.

Incubations. Methapyrilene, NADPH, NADP, glucose 6-phosphate, and glucose-6-phosphate dehydrogenase were purchased from Sigma Chemical Co. ^{14}C-Labeled sodium cyanide (50–60 mCi/mmol) was purchased from New England Nuclear and was diluted to the desired specific activity (0.25 mCi/mmol). Most incubations were performed with suspensions of rabbit liver 100000*g* microsomes prepared at 0–4 °C in a cold room. Male Dutch rabbits 6 months to 1 year old were fasted for 24 h and then stunned and decapitated. The livers were excised and rinsed in cold isotonic (1.15%) KCl. The liver tissue (12.5 g) was homogenized in 25 mL of cold 0.2 M pH 7.4 Tris-HCl buffer using a Potter-Elvehjem Teflon pestle homogenizer. The homogenate was centrifuged at 10000*g* for 20 min in a Sorvall RC-2 refrigerated centrifuge at 0–4 °C to yield the 10000*g* supernatant fraction. The supernatant fraction was recentrifuged at 100000*g* for 1 h at 0–4 °C in a Spinco Model L refrigerated centrifuge to obtain the microsomal pellet. The sediment (microsomal pellet) was resuspended in 25 mL of cold 0.2 M pH 7.4 Tris-HCl and the suspension was then recentrifuged at 100000*g* for 1 h at 0–4 °C. The resultant pellet was then resuspended in 25 mL of cold 0.2 M pH 7.4 Tris-HCl for use in incubations. A similar procedure was followed when rat microsomal preparations were used. Male Sprague–Dawley rats weighing 200–250 g were used. In this case, the animals were treated for 3 consecutive days with phenobarbital (4 mg/kg ip), prior to the 24-h fasting period and sacrifice.

A typical incubation (5.0 mL total volume) contained 4 mL of the microsomal fraction (corresponding to 0.5 g of liver/mL), $MgCl_2$ or $MgSO_4$ (15 mM), methapyrilene (1 mM), and NADPH (4 mg every 20 min) or an NADPH-generating system (0.4 mM NADP, 7.5 mM glucose 6-phosphate, 0.5 units/mL glucose-6-phosphate dehydrogenase, 5.0 mM $MgCl_2$). Incubations in the presence of sodium cyanide contained 1.0 mM NaCN or $Na^{14}CN$ (in H_2O); the specific activity of the $Na^{14}CN$ was adjusted such that the amount of radiolabel in a 5-mL incubation was about 5×10^4 dps. Incubations were performed at 37 °C in a metabolic shaker in air for 1 h.

Postincubation mixtures for radioactivity assays were adjusted to pH 10 by the addition of solid K_2CO_3 and extracted with CH_2Cl_2 (2 × 6 mL). The CH_2Cl_2 extracts were washed once with 5 mL of saturated K_2CO_3 solution, and the resulting aqueous wash was back-extracted with CH_2Cl_2 (2 × 3 mL). When necessary, phase separation was facilitated by centrifugation. The combined organic extract was dried over K_2CO_3 and evaporated to dryness under an N_2 stream at 25–30 °C (N_2 gas conducted through Teflon tubing). The residue was taken up in 10 mL of Aquasol scintillation fluid and counted in a liquid scintillation counter. Timed 1-mL aliquots from incubations for the quantitation of methapyrilene metabolism were made basic with solid K_2CO_3 and were then chilled in ice. The internal standard pyrilamine (maleate salt, 200 μL of a 10.0 mg/mL aqueous stock solution) was added immediately to each aliquot, and the resulting mixture was extracted with CH_2Cl_2 (3 × 6 mL). The combined CH_2Cl_2 extract was dried over K_2CO_3, evaporated to near dryness under N_2 (25–30 °C), reconstituted to 0.3 mL with CH_2Cl_2, and analyzed by GC (3% OV-25; column oven temperature 150 °C isothermal for 2 min 40 s, and then programmed at 6 °C/min to 250 °C). Retention times were as follows: methapyrilene, 10.6 min; normethapyrilene, 12.0 min; pyrilamine, 14.8 min. Qauntitation of GC peaks was achieved by measuring peak heights.

For preparative studies, 64 5-mL postincubation mixtures were combined and extracted in 3 equal aliquots with 3 × 200 mL CH_2Cl_2 each. The combined organic extract was concentrated under vacuum to about 50 mL and was washed once with 10% NaCl (10 mL). The CH_2Cl_2 layer then was extracted with 20 mL of 2 N HCl, and the pH of the aqueous layer was adjusted to 11 by the slow addition of 4 N NaOH with external cooling. The basic solution was extracted with CH_2Cl_2 (3 × 25 mL), which was dried (K_2CO_3) and rotary evaporated to dryness. The crude extract could be purified by preparative TLC (2000 μm silica gel plates) preeluted twice with 1:1 benzene/MeOH and reactivated by heating at 120 °C prior to use with $CH_2Cl_2/CH_3CN/Et_3N$ (90:10:1) as the developing solvent. The desired radioactive zone (by radioscan and UV, R_f 0.55) was scraped and extracted with 1:1 CH_2Cl_2/CH_3CN. The organic extract was dried over K_2CO_3 and evaporated to near dryness to yield compound 18, which was identified by GC, GC–EIMS, and NMR analysis and by comparison with synthetic 18.

Several attempts were made to extract polar metabolic products after the initial extraction of the preparative incubation mixture. The aqueous layer was treated with 4 N HCl such that the pH of the solution was adjusted sequentially to 8, 7, 6, 5, and 4, and each mixture was extracted with CH_2Cl_2 (3 times) or by continuous extraction for 24 h. The organic extracts were dried over K_2CO_3, evaporated, and analyzed by GC or GC–EIMS. The resiudes also were treated with diazomethane (in MeOH), and the reaction mixtures were analyzed by GC–EIMS.

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