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IN VITRO METABOLISM OF CHLORPHENIRAMINE IN THE RABBIT

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Abstract—The *in vitro* metabolism of 3-(*p*-chlorophenyl)-3-(2-pyridyl)-*N*,*N*-dimethylpropylamine (chlorpheniramine, I) by rabbit liver microsomes was examined. The metabolites, tentatively identified by gas–liquid chromatography–mass spectrometry, included the mono- and didemethyl metabolites, the aldehyde that results from deamination, and further metabolites of this aldehyde including its intramolecular cyclization product, an indolizine, and its reduction product, the alcohol. Inhibition of metabolism of I by N_2, CO, SKF-525A, 2,4-dichloro-6-phenylphenoxyethylamine (DPEA), or deletion of NADPH implies some involvement of cytochrome P-450 in the metabolic reactions. Quantitation of metabolism in these studies accounted for only 69% of the dose, so that binding and/or other undetected metabolic pathways were operative.

Chlorpheniramine, [3-(*p*-chlorophenyl)-3-(2-pyridyl)-*N*,*N*-dimethylpropylamine], I (Scheme 1), is an old antihistaminic drug found in many over-the-counter formulations. Although it is widely used, available metabolic information is somewhat limited.

Early studies demonstrated that demethylation of I to form both the monodemethyl metabolite (II, scheme 1) and the didemethyl metabolite, III, is facile [1–4]. However, *in vivo* metabolic experiments with labeled I in the rat [1, 3], dog [1, 3] and human [4] revealed a large percentage of the dose to be unidentified polar metabolite(s). Osterloh *et al.* [5] identified both 3-(*p*-chlorophenyl)-3-(2-pyridyl)-propionic acid, VII, and 3-(*p*-chlorophenyl)-3-(2-pyridyl)-propyl alcohol, V, as *in vivo* metabolites of I in the dog [5], but they also reported that at least one other unidentified metabolite was observed during analysis. In rabbits [6], only 2.9% of the dose of I is accounted for in the urine by I, II, and III, again suggesting the presence of a large percentage of unidentified metabolite(s). Another *in vitro* study of the metabolism of I by rabbit liver microsomes [7] reported two unidentified metabolites of high polarity.

In preliminary reports from this laboratory, we have shown that the *in vitro* metabolism of I in both rat [8] and rabbit [9] liver microsomes produces not only II and III, but also the alcohol V as well as unknown metabolites. Since we found that metabolic conversion of I to metabolites was much less in the rat than in the rabbit, the goals of this study were to investigate the metabolism of I in rabbit liver microsomes, to identify any unknown metabolites, and to determine whether or not the metabolism of I is mediated by any cytochrome P-450 isozymes.

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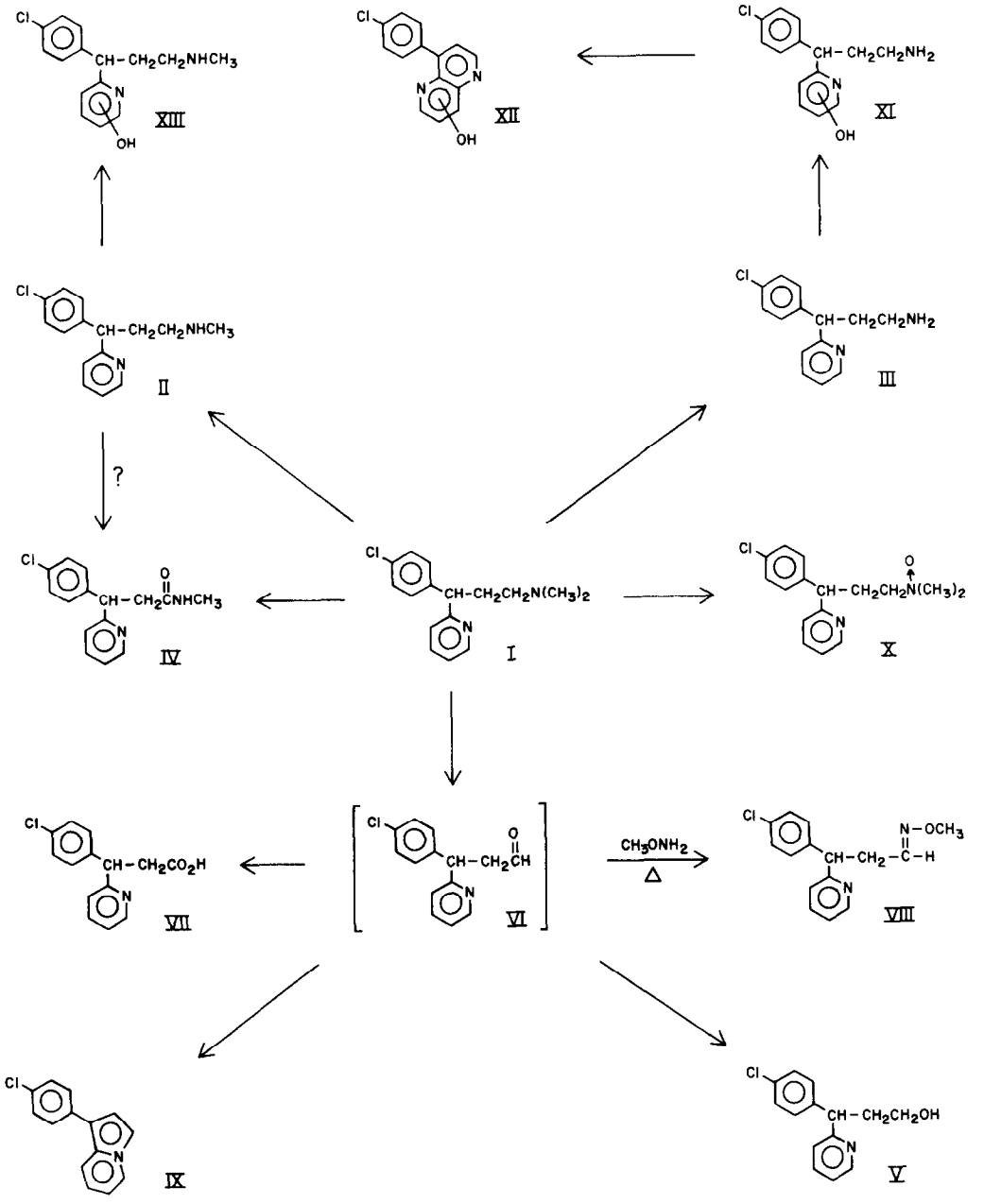
MATERIALS AND METHODS

Materials. Chlorpheniramine maleate was donated by Berlex Laboratories, Cedar Knolls, NJ. SKF-525A, 2-*p*-chloro-α-(2-aminoethylbenzyl)pyridine, III, and 2-*p*-chloro-α-(2-methylaminoethylbenzyl)-pyridine, II, were obtained from Smith Kline & French, Philadelphia, PA. DPEA (2,4-dichloro-6-phenylphenoxyethylamine) was a gift of the Eli Lilly Co., Indianapolis, IN. Benzphetamine·HCl was obtained from Upjohn Laboratories, Kalamazoo, MI. Monosodium glucose-6-phosphate, glucose-6-phosphate dehydrogenase, and nicotinamide dinucleotide phosphate were purchased from the Sigma Chemical Co., St. Louis, MO. Phenobarbital was purchased from the UCLA pharmacy. Methoxylamine hydrochloride was purchased from the Fisher Chemical Co., Tustin, CA. MBTFA [*N*-methyl-bis(trifluoroacetamide)], BSTFA [bis(trimethysilyl)-trifluoroacetamide], HFBI (heptafluorobutyryl-imidazole), and TMCS (trimethylchlorosilane) were obtained from the Regis Chemical Co., Gardena, CA. MTBSTFA [*N*-methyl-*N*-(*tertiary*-butyldimethylsilyl)trifluoroacetamide was purchased from the Pierce Chemical Co., Rockford, IL.

Animals and tissue preparation. New Zealand white male rabbits (2 to 2.5 kg) were purchased from Red Bean Farms, Redlands, CA. When pretreated with phenobarbital, injections were given in isotonic saline for 3 days, i.p. at 60 mg/kg/day. Sacrifice was by stunning and then exsanguination. The liver was removed and rinsed in ice-cold isotonic KCl; 10 g pieces were weighed and then homogenized in 30 ml of isotonic KCl. The homogenate was centrifuged at 9000 *g* to sediment nuclei and cellular debris. The supernatant fraction was transferred to clean ultracentrifuge tubes and spun for 1 hr at 105,000 *g*. The resulting pellet was washed once by homogenization



Scheme 1. Metabolic pathways of chlorpheniramine and chemical conversions discussed in this paper.

in fresh isotonic KCl and recentrifuged for 0.5 hr to yield a microsomal pellet. The pellet was stored at −80° until used (but not for more than 1 week).

Incubations. The contents of a typical incubation tube consisted of: 0.5 ml I (final concentration of 1.0 mM), 0.5 ml of an NADPH-generating system (30 μmol monosodium glucose-6-phosphate, 12 μmol magnesium chloride, 2.6 μmol $NADP^+$ and 8 units of glucose-6-phosphate dehydrogenase), 1.0 ml microsomal suspension equivalent to 0.33 g liver, 1.0 ml (0.5 M) potassium phosphate buffer, pH = 7.4, and distilled water (or inhibitor solution as needed) to make up a final volume of 5 ml. Experiments were run in triplicate at 37° for 0, 10, 20, 30, and 60 min incubation periods, with each time point in duplicate.

Inhibitor incubations. A variety of conditions were utilized to facilitate studies of the metabolism of I with various substances. Nitrogen or $CO:O_2$ coincubation experiments utilized the appropriate gas gently purged through a syringe needle into the flask for 5 min prior to addition of substrate along with gentle agitation of the flasks, and further gas introduction during the incubation period as well. SKF-525A was added to an incubation flask for a final concentration of 0.1, 0.5 or 1.0 mM, whereas DPEA

was added for final concentrations of 0.001, 0.010, or 0.100 mM.

Assays. Metabolic incubations were terminated by pouring the incubation mixture into 40-ml extraction tubes containing 10 ml of ice-cold methylene chloride and 1.0 μmol benzphetamine·HCl, as the internal standard. The tubes were shaken for 20 min and centrifuged at 2900 rpm for 10 min; the upper aqueous phase was discarded. An 8.0-ml aliquot of the organic phase was transferred to clean 12-ml conical tubes and evaporated under a nitrogen stream to a final volume of 0.1 ml. For routine quantitation, trifluoroacetyl derivatives were prepared by adding 0.1 ml MBTFA reagent to each experimental tube and heating at 60° for 1 hr. Other derivatizing agents were utilized in the same amount, with identical derivatization conditions to prepare their respective derivatives. Samples were analyzed isothermally at 195° by gas–liquid chromatography using a Hewlett–Packard 5840 GLC, equipped with a flame ionization detector and a (6 ft, 2 mm i.d.) glass column packed with 3% SE-30 on 100/120 GCQ. Injector and detector temperatures were both set at 250°. The order of elution of I and metabolites from this column was benzphetamine (internal standard), I, V, IV, III, II, IX and XII (see Fig. 1). Several metabolic experiments were analyzed on a Hewlett–Packard 5880A gas–liquid chromatograph equipped with a 12 m OV-1 fused silica capillary column (0.32 mm i.d. and 0.52 μm film thickness) in order to try to quantitate metabolites, which were both formed at very low levels and often obscured by endogenous GLC peaks, when analyzed by the packed GLC column (e.g. V, IX and XII).

GLC–Mass spectrometry. For routine GLC–mass spectrometry analysis, a 12 m fused silica Hewlett–Packard OV-1 column (0.22 mm i.d. and 0.33 μm film thickness) was connected to a Hewlett–Packard 5987 mass spectrometer operating in the splitless, capillary direct mode. The ionizing voltage was set at 70 eV, and the injector, interface, analyzer and source temperatures were 275, 300, 240 and 200° respectively. Data reduction was carried out by an RTE VI operating system connected to a Hewlett–Packard 1000 Computer.

For exact mass measurements, gas–liquid chromatography–mass spectrometry was performed at the University of California at Riverside, Department of Chemistry regional mass spectrometry facility. Conditions utilized were as follows. A VG 7070E-HF mass spectrometer was interfaced with a Hewlett–Packard 5790A gas–liquid chromatograph, which was fitted with a 30 m fused silica DB-1 capillary column (0.52 μm film thickness and 0.33 mm i.d.). Data reduction was by a PDP-11-34 computer system equipped with a VG operating system and associated software. Electron impact mode was used at 70 eV with the internal reference for exact mass measurements being perfluorokerosene (PFK). Chemical ionization conditions utilized ultrapure methane as the reagent gas.

Trapping experiments. To detect any possible metabolic formation of an aldehyde in a manner similar to that of our earlier report on trapping an aldehyde intermediate of phencyclidine metabolism [10], an incubation of 1 mM I with rabbit liver microsomes was quenched by transferring it to extraction tubes containing 100 mg methoxylamine hydrochloride and heating it in a water bath at 100° for 30 min. Under these conditions most aldehydes formed are converted to *O*-methyloxime derivatives. These samples were then extracted as for other incubations and were analyzed by GLC–mass spectrometry, with a different GLC temperature program designed to resolve any apparent overlapping GLC peaks observed in the initial experiment. These conditions were: 195° for 4 min, then a 16°/min increase to 260°, and maintenance at 260°.

Identification of unknowns. Sets of incubation extracts of I and liver homogenates were derivatized with MBTFA, HFBI, BSTFA-TMCS or MTBSTFA to form the trifluoroacetyl, heptafluorobutyryl, trimethylsilyl, and *tertiary*-butyldimethylsilyl derivatives, respectively, in order to tentatively identify any unknown GLC peaks present. In addition, the presence of a chlorine atom in the mass fragment ions observed in an unknown (indicated by double peaks separated by 2 a.m.u. in the ratio of 3:1) was taken as evidence of a GLC peak being derived either from I, or a metabolite of I.

Quantitation of metabolites. Standard curves were constructed by adding known amounts (0.1 to 5.0 μmol, I; and 0.01 to 0.50 μmol, II and III) of pure compound to incubation mixtures at 0° and extracting them in the presence of benzphetamine (1.0 μmol), the internal standard. The measured peak height ratios (to internal standard against amounts added) were then plotted. Standard curves were linear over the concentration ranges chosen, which were appropriate for the metabolic results obtained. The standard curve for II was used to estimate the amounts of IX and XII (reference standards were not available) because, both IX and XII elute from the GLC column closest to II, and thus have similar polarities, Statistically significant differences from control incubation values were determined using Student's two-tailed *t*-test, with $P < 0.05$ considered significant.

RESULTS

When chlorpheniramine (I, scheme I) was incubated with rabbit liver microsomes, six metabolites were detected by GLC or GLC–mass spectrometry. The initial GLC temperature program separating these compounds from I and the internal standard was utilized for mass spectrometric analysis of an incubation extract, which was derivatized with MBTFA, and the results were plotted as total ion current versus time (Fig. 1). That all of the GLC peaks were metabolites of I was shown by the time, protein and NADPH dependence of their formation, as well as by the fact that each of these GLC peaks produced mass fragments containing chlorine (identified by the characteristic 3:1 ratio of two ions separated by 2 a.m.u., due to the two naturally occurring stable isotopes of chlorine).

Identification of metabolites. Methylene chloride extracts of incubation mixtures of rabbit liver microsomes with I were evaporated, derivatized with various derivatizing agents (MBTFA, HFBI, BSTFA, or MTBSTFA), and injected into the GLC–MS system. The retention times and mass spectral fragmentation

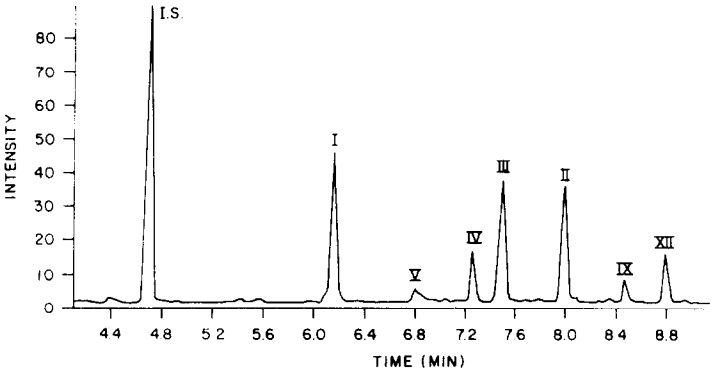


Fig. 1. Gas–liquid chromatographic separation of chlorpheniramine, I, from the internal standard (benzphetamine, I.S.) and metabolites formed (II, III, IV, V, IX and XII of Scheme 1) in a rabbit liver microsomal incubation experiment.

patterns of authentic I, II, and III were identical to those obtained from metabolic sample extracts (Table 1).

The tentative identification of metabolite V (Fig. 1) as the deaminated alcohol (Scheme 1) is based upon the mass spectra (Table 1) obtained. The trifluoroacetyl derivative of V showed a major ion at m/z 227 due to loss of trifluoroacetic acid plus H_2 from the parent ion (which fully aromatizes the resulting ion), and has been reported in the spectrum of authentic underivatized V [5].

The trimethylsilyl derivative of V exhibited an ion for the loss of a methyl group, and the *tertiary*-butyldimethylsilyl derivative yielded the expected large ion for P-57 a.m.u., as well as an ion for P-15 a.m.u.

The amide, IV, was tentatively identified by the fact that its mass spectrum was very similar to that obtained for I (same parent ion of m/z, 274), but the GLC retention time of IV was considerably larger than that for I, indicating increased polarity relative to I. Also, an additional GLC peak appeared at a *much* longer retention time, after derivatization with MBTFA, whose mass spectrum was consistent with the same amide structure, but as the trifluoroacetyl derivative (Table 1).

Tentative identification of the GLC peaks labeled IX and XII in Fig. 1 initially posed a problem, since

Table 1. Mass spectra of chlorpheniramine metabolites

Compound	m/z		
	Parent ion	Base ion	Other ions
II*	356(1)	216	287(2), 259(3), 230(10), 203(60), 167(50), 140(25), 78(15), 69(25)
III*	342(1)	216	273(3), 246(2), 230(7), 203(60), 167(55), 139(10), 78(20), 69(25)
IV*	370(3)	216	273(4), 244(3), 203(25), 167(45), 78(20), 69(30)
IV†	274(2)	216	203(50), 167(50), 78(20)
V*	343(0)	227	230(10), 191(45), 69(15)
V‡	319(1)	203	304(9), 216(45), 167(30), 75(25), 73(20)
V§	361(0)	75	346(15), 304(85), 230(40), 203(20), 167(35)
IX*	323(60)	191	288(5), 254(80), 226(60), 95(50), 69(25)
IX†	227(100)	227	229(32), 191(35), 95(20)
IX‖	423(80)	254	226(45), 191(80), 69(20)
XI‡	334(0)	167	319(25), 245(60), 202(90), 75(20)
XI§	376(0)	167	361(4), 319(35), 245(45), 202(95), 75(50)
XII*	352(35)	255	317(4), 283(2), 227(35), 191(15), 69(25)
XII‖	452(30)	255	227(20), 69(20)
XIII‡	348(0)	167	333(25), 259(70), 230(25), 202(85)
XIII§	390(0)	202	375(5), 333(25), 315(8), 259(35), 230(25), 167(30)

Mass spectra were obtained under the conditions described in Materials and Methods. Derivatizations were also described in that section.

* Trifluoroacetyl derivative.
† Underivatized.
‡ Trimethylsilyl derivative.
§ *Tertiary*-butyldimethylsilyl derivative.
‖ Heptafluorobutyryl derivative.

Table 2. Mass spectrometric investigations of metabolites IX* and XII*

Metabolite	C.I.† Parent ion	Measured ion (m/z)	Formula	Calc'd for	Found‡
IX	324	323§	$C_{16}H_9NOClF_3$	323.0325	323.0302
IX	324	254‖	$C_{15}H_9NOCl$	254.0373	254.0251
XII	353	352§	$C_{16}H_8N_2O_2ClF_3$	352.0226	352.0372
XII	353	257¶	$C_{14}H_8N_2OCl^{37}$	257.0296	257.0236

* As trifluoroacetyl derivatives, and analyzed as described in Materials and Methods.
† Chemical ionization mass spectroscopy (methane) gave mH^+ ions (P+1) as predicted.
‡ Exact mass measurements as described in Materials and Methods.
§ Highest mass observed under 70 ev, electron impact.
‖ Base peak in spectrum.
¶ m/z 257 is the ion containing Cl^{37} (base ion = m/z 255) which was chosen for analysis because m/z 255 is a prominent ion in the mass spectrum of perfluorokerosene, utilized as the reference standard for exact mass measurement, resulting in a large m/z 255 background signal.

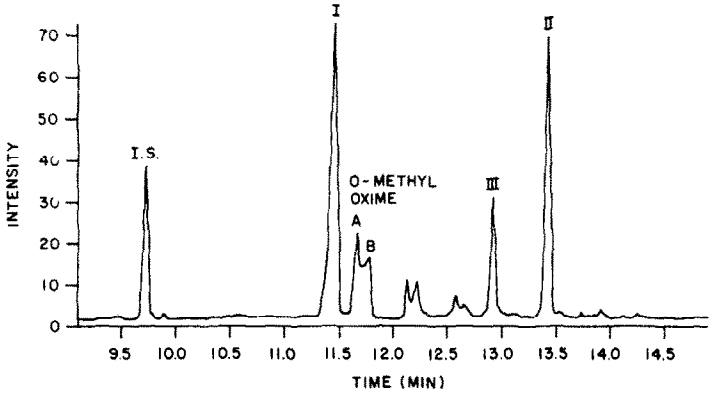


Fig. 2. Gas–liquid chromatogram resulting from analysis of an incubation extract of rabbit liver microsomes and chlorpheniramine, treated *in situ* with methoxylamine.

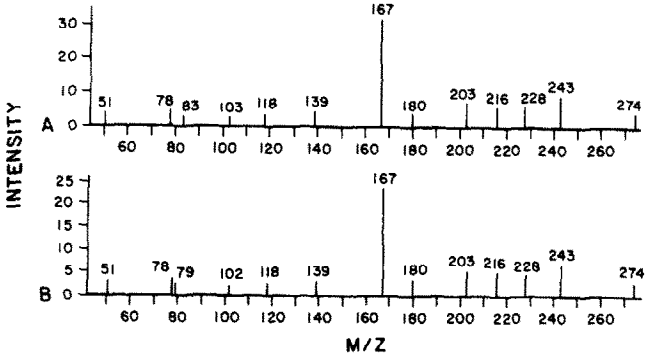


Fig. 3. Electron impact mass spectra of peaks A and B from the chromatogram of Fig. 2, showing the isomeric identity of these two peaks with a molecular weight of m/z 274 and tentatively identified as the syn and anti methoximes of β-(2-pyridyl)-β-(*p*-chlorophenyl)propionaldehyde, VIII (Scheme 1).

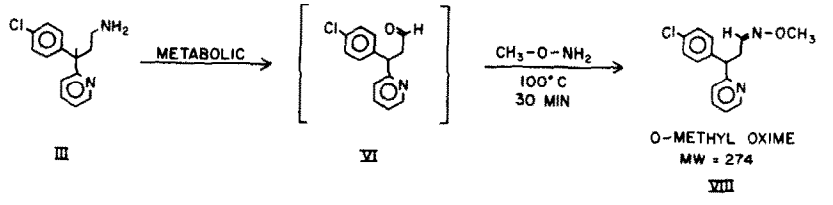


Fig. 4. Schematic representation of the metabolic formation of the intermediate aldehyde, VI, and trapping *in situ* by methoxylamine.

the mass spectra of their trifluoroacetyl derivatives (Table 1) were not consistent with any hypothetical metabolites of I, yet the peaks were clearly derived from I, because they contained chlorine, and their formation was time, protein, and NADPH dependent. Mass spectra of the heptafluorobutyryl derivatives of IX and XII (Table 1) were very similar to those obtained for the trifluoroacetyl derivatives, except that many of the ions formed were 100 a.m.u. larger, due to the addition of two more-CF_2-groups in the derivatives formed. Trimethylsilylation of an incubation extract of I and liver microsomes (with BSTFA), followed by GLC–MS monitoring of the resultant GLC peaks revealed that the mass spectrum of the first non-volatile unknown under these conditions corresponded to structure IX but without derivatization (Table 1). Scanning of the second GLC peak yielded a mass spectrum which corresponded to XI (a hydroxypyridyl derivative of metabolite III). Indeed, under conditions of derivatization with MTBSTFA, the corresponding *tertiary*-butyldimethylsily derivative of XI was detected as well as underivatized IX, but neither XII nor derivatized IX could be found. In addition, another GLC peak was detected under these silylation conditions (identified as a hydroxypyridyl derivative of II labeled XIII; see Table 1). However, the relative peak size of this metabolite was always small.

To further confirm the tentative identifications of IX and XII, both exact mass measurements on a high resolution mass spectrometer, and chemical ionization mass spectrometric data were obtained so that further characterization of the structures of IX and XII was possible. Methane chemical ionization mass spectra of both IX and XII as their trifluoracetyl derivatives gave the characteristic MH^+ ions (or parent + 1 a.m.u.) consistent with their assigned structures (Scheme 1). Exact mass measurements of both the base and parent ions were consistent with the empirical formulas which are required for structures IX and XII (Table 2).

Final positive confirmation of the identification of these metabolites still awaits both the availability of synthetic reference standards and subsequent analytical comparisons with the metabolically produced products.

Trapping experiments. Incubations of liver microsomes and I which were poured immediately into extraction tubes containing methoxylamine-hydrochloride, heated for 1 hr, and analyzed by GLC–mass spectrometry produced a total ion current chromatogram like that depicted in Fig. 2. Mass spectral scanning of the earliest eluted pair of partially resolved (or double) peaks yielded two identical mass spectra (Fig. 3) which corresponded in molecular weight (m/z 274) and fragmentation pattern to that expected for structure VIII (Scheme 1). Such a molecule would result from the trapping of intermediate VI (Scheme 1) by methoxylamine. Oxime formation of such an aldehyde would form both syn and anti isomers of the resultant oxime, which then partially separate on the GLC column. The proposed reaction sequence is summarized in Fig. 4.

Requirements of the microsomal metabolism of chlorpheniramine. A summary of the effects of various substances on the metabolism of I is presented

Table 3. Effect of various substances on the microsomal metabolism of chlorpheniramine, I

Substances	Metabolite formation (% of control)*					Consumption of I
	II	III	IV	IX	XII	
Complete	100 ± 5	100 ± 2	100 ± 17	100 ± 7	100 ± 30	100 ± 9
—NADPH generating system	0	0	0	0	0	0
N_2	33.2 ± 11.8†	5.6 ± 1.6†	8.1 ± 3.9†	0	0	51.3 ± 28.4
$CO:O_2$ (4:1)	108.3 ± 4.3	74.4 ± 7.6‡	71.1 ± 11.0	55.6	20.5	83.4 ± 3.5
DPEA (1 μM)	86.4 ± 9.7	73.2 ± 20.2	70.7 ± 15.3	102.8	48.7	98.2 ± 22.6
DPEA (10 μM)	72.8 ± 9.0	37.5 ± 8.5	36.3 ± 4.2	36.1	0	60.7 ± 7.7
DPEA (100 μM)	14.6 ± 0.8§	0†	48.4 ± 21.6	55.6	20.5	17.0 ± 11.5‡
SKF-525A (0.1 mM)	93.5 ± 7.8	57.7 ± 6.5	46.5 ± 12.2‡	158.3	20.5	83.5 ± 11.4
SKF-525A (0.5 mM)	86.9 ± 8.8	31.4 ± 4.9‡	30.4 ± 13.0‡	102.8	2.6	38.7 ± 8.1‡
SKF-525A (1.0 mM)	73.3 ± 31.8‖	3.4 ± 1.5†	22.8 ± 11.5†	0	0	39.3 ± 4.1†
Phenobarbital (4 × 60 mg/kg. i.p.)	142.3 ± 7.4†	133.8 ± 18.4‖	98.8 ± 11.8	100	192.3 ± 53.3	132.7 ± 10.4

* Control conditions: incubation of I (initially 1 mM) for 60 min in rabbit liver microsomes (0.33 g liver equivalent of microsomes) and an NADPH-generating system. Recovery, workup and analysis are described in Materials and Methods. Values are mean ± SEM, N = 6.
† $P < 0.01$. ‡ $P < 0.05$. § $P < 0.001$. ‖ $P < 0.10$.

Table 4. Levels of chlorpheniramine and metabolites*

Incubation time (min)	I	II	III	IV†	IX‡	XII‡	Total (mass balance)
0	4.97 ± 0.55	0	0	0	0	0	4.97 ± 0.55
30	3.45 ± 0.10	0.84 ± 0.10	0.28 ± 0.04	0.05 ± 0.01	0.02 ± 0.00	0.01 ± 0.00	4.65 ± 0.27
60	2.72 ± 0.15	0.84 ± 0.07	0.53 ± 0.03	0.11 ± 0.02	0.04 ± 0.01	0.04 ± 0.01	4.28 ± 0.29

* Levels were measured by gas–liquid chromatography as described in Materials and Methods, produced in a rabbit liver microsomal incubation. Values are means ± SEM, N = 6.

† Levels were quantitated using the standard curve for I since a synthetic reference standard was not available.

‡ Levels were quantitated using the standard curve for II since synthetic reference standards were not available.

in Table 3. Elimination of the NADPH-generating system (or the microsomal protein, data not shown) shut off both the consumption of I and the appearance of metabolites. Replacement of the incubation atmosphere by either nitrogen or carbon monoxide:oxygen (4:1) decreased the appearance of metabolites, indicating some involvement of the cytochrome P-450 monooxygenase in the metabolism of I. Both SKF-525A and DPEA (cytochrome P-450 inhibitors) inhibited the formation of metabolites as well as the consumption of I by microsomal enzymes, again indicating some involvment of cytochrome P-450 monooxygenase in the metabolism of I.

Metabolism. Levels of the various metabolites of I formed are presented in Table 4. Amounts of both V and XIII (Scheme 1) are not included, because these levels were always very low and thus subject to wide variability. Table 5 summarizes the percentage of consumed chlorpheniramine, I, that is accounted for by the measured metabolites, II, III, IV, IX, and XII and indicates that only 69% of the I metabolized had been detected after a 60-min incubation period.

DISCUSSION

Several new metabolites or metabolic pathways of I are described in these experiments, including the deamination of I to form the aldehyde, VI (Scheme 1), which is a viable metabolic route for several reasons. First, the metabolic reduction of VI yields V (Scheme 1) which is detected directly by GLC–MS analysis in incubation extracts, albeit at very low levels. Second, the aldehyde, VI, has been trapped via the *in situ* formation of its *O*-methyloxime derivative (Figs. 2–4), subsequent gas–liquid chromatographic separation of the syn and anti isomers, and mass spectrometric identification. Third, *a priori*, the aldehyde, VI (Scheme 1) should be extremely unstable chemically and would be expected to quickly cyclize to the 1(*p*-chlorophenyl)pyrrocoline (also called an indolizine). In fact, a synthesis of β-(2-pyridyl)propionaldehyde [the identical compound to VI (Scheme 1) but without the *p*-chlorophenyl-substituent] has been reported [11], and this compound spontaneously cyclizes to the corresponding pyrrocoline. A later study of methods to synthesize several different pyrrocolines found the same result, namely, spontaneous cyclization of the pyridylpropionaldehyde derivatives to form the corresponding pyrrocolines [12]. Thus, the tentative identification of the indolizine, IX, in this case, is not only not surprising but would be expected. Acylation of IX during derivatization with MBTFA occurred readily, yielding the trifluoroacetyl derivative of IX, which was then subsequently identified by exact mass measurements (Table 2). Since the reactivity of an acylating agent depends on the electrophilicity of the acylating atom, it would be expected that acylation of the aromatic nucleus of IX would be much more facile for the trifluoroacetyl-ion than for the trimethylsilyl-ion. Such is found to be the case since, under conditions employed for trimethylsilylation of metabolic extracts with BSTFA, only underivatized (unacylated) IX can be detected by GLC–mass spectrometry.

It appears that the acylation conditions of derivatization with MBTFA promote the formation of XII (namely, 4-[*p*-chlorophenyl]-7-hydroxy-1,5-naphthyridine), whereas the milder conditions of silylation produce only derivatives of XI. Thus, the chemical cyclization of XI to XII is promoted by derivatization with MBTFA, and XI is the true (albeit tentatively identified) metabolite of I under these metabolic conditions. The presence or absence of XIII in any experiment is not considered significant in reference to cyclization of XI or XII, since levels of XIII, when detected at all, are always extremely low and subject to wide variability.

Phenobarbital pretreatment of the animals increased the formation of some metabolites (Table

Table 5. Percentage of chlorpheniramine, I, metabolized that each metabolite represents*

Incubation time (min)	I Metabolized (μmol)	II	III	IV	IX	XII	Total
30	1.52	55.3	18.4	3.3	1.3	0.7	79
60	2.25	37.3	23.6	4.9	1.8	1.8	69

* Results are from a rabbit liver microsomal incubation, in phosphate buffer with a NADPH-generating system, as described in Materials and Methods.

3), and barbiturates are known to increase the amount of some isozymes of cytochrome P-450 in the liver. Inhibition of some metabolite formation by the presence of nitrogen, carbon monoxide, or omission of an NADPH-generating system is consistent with the involvement of cytochrome P-450 in the metabolism of I. In addition, potent inhibition of the metabolism of I by either SKF-525A or DPEA also indicates the involvement of cytochrome P-450 in the metabolism of I. SKF-525A has been shown to be an active inhibitor of several metabolic transformations of drugs [13, 14], and DPEA has been found to bind to both soluble and microsomal cytochrome P-450 and to competitively inhibit the interaction of substrates with cytochrome P-450 [15].

The levels of metabolites IV, IX and XII (Table 4) were not high, when compared to those of either II or III (even if their GLC responses were relatively low in these approximate quantitations), and they would not account for all of the metabolized I (Table 5). This suggests that there are metabolic pathways not measured in this study and/or binding of VI or other reactive metabolites to tissues.

In summary, the metabolism of chlorpheniramine, I, in the rabbit, was found to be mediated, at least in part, by hepatic cytochrome P-450. The formation of the deamination metabolite, VI was shown to be a viable, metabolic pathway, since VI had been trapped *in situ* via oxime formation and both the cyclization and reduction products of VI (namely IX and V) were detected in incubation extracts. Further metabolic work needs to be done to address the question of the approximately 30% of the metabolized I that is unaccounted for.

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