Microbial Transformation of the Antihistaminic Drug Triprolidine Hydrochloride

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Received June 23, 1987, from the *Department of Health and Human Services, Food and Drug Administration, National Center for Toxicological Research, Jefferson, AR 72079.* Accepted for publication October 6, 1987.

Abstract □ The production of a known mammalian metabolite of the antihistamine triprolidine through fungal metabolic transformation has been demonstrated. The filamentous fungus *Cunninghamella elegans* ATCC 9245 was grown in Sabouraud dextrose broth containing triprolidine hydrochloride monohydrate. One major metabolite was extracted with methylene chloride, isolated by high-performance liquid chromatography, and identified by its proton-nuclear magnetic resonance and desorption chemical ionization mass spectral properties as hydroxymethyl triprolidine (2-[1-(4-hydroxymethylphenyl)-3-(1-pyrrolidinyl-1-propenyl)] pyridine). After 240 h of incubation, the hydroxymethyl derivative represented ~55.0% of the initial dose. Fungal oxidation of hydroxymethyl triprolidine to the corresponding carboxylic acid triprolidine derivative (also a known mammalian triprolidine metabolite) was not observed. No mutagenic activity was observed for triprolidine and hydroxymethyl triprolidine by reversion of *Salmonella typhimurium* strains TA97, TA98, TA100, and TA104 at concentrations up to 1000 and 200 μg/plate, respectively. These results suggest that the fungal metabolism of triprolidine to the hydroxymethyl derivative occurs predominantly through pathways which do not result in mutagenic activation. Incubation of *C. elegans* with triprolidine under an $^{18}O_2$ atmosphere and subsequent electron impact mass spectral analysis of the hydroxymethyl triprolidine formed indicate that molecular oxygen was incorporated into the methyl group and suggest a mono-oxygenase catalyzed reaction. This study parallels previous studies on the mammalian metabolism of triprolidine and clearly indicates that the microbial transformation of triprolidine is a useful alternative for the synthesis of potential mammalian metabolites.

Increased interest in the toxicology of common over-the-counter antihistamines occurred after the 1980 report of 100% liver tumor incidence induced in rats exposed to the widely used antihistamine methapyrilene.[1] Recent research efforts have sought to determine how methapyrilene or its metabolites initiate the carcinogenic process.[2-5] Many microorganisms can metabolize certain compounds in a manner similar to that of mammalian enzyme systems.[6] Recently, we applied microbial transformation methodologies to study the fungal metabolism of methapyrilene and other structurally similar antihistamines.[7,8] These studies produced antihistamine metabolites that were identical to metabolites identified during in vivo and in vitro experimental animal studies.[9,10]

Triprolidine is an alkylamine-type antihistamine that is currently widely used by the general public in over-the-counter tablet and syrup formulations. Data concerning the bioavailability and disposition of triprolidine in experimental animals and humans have been reported.[11-13] Metabolism data reported for triprolidine, however, is limited. Kuntzman et al.[14] have reported that triprolidine is metabolized by guinea pig liver microsomes in the presence of an NADPH-generating system to form hydroxymethyl triprolidine. They also found that incubation of triprolidine with guinea pig liver cytosol produced the corresponding carboxylic acid.

The high degree of public exposure to triprolidine and the paucity of toxicological information concerning the antihistamine and its known metabolites has resulted in triprolidine being nominated for toxicological bioassay studies using laboratory animals by the National Toxicology Program. The aim of the present study was to microbially produce sufficient quantities of triprolidine metabolites for structure elucidation and to determine their mutagenic potential. This information will be useful to other proposed toxicological studies of triprolidine.

Experimental Section

Chemicals—Triprolidine hydrochloride monohydrate (2-[1-(4-methylphenyl)-3-(1-pyrrolidinyl-1-propenyl)] pyridine hydrochloride monohydrate) was purchased commercially (Chemical Dynamics, South Plainfield, NJ). Detailed chemical purity (100%) data of this compound has been reported.[15] All solvents were of HPLC grade. Deionized water (18 mohms/cm) was obtained from a Milli-Q-Water Purification System (Waters Associates, Milford, MA). All other chemicals were of reagent grade and of the highest purity available.

Microbial Culture and Biotransformation Procedures—Cultures of *Cunninghamella elegans* ATCC 9245 were maintained on Sabouraud dextrose agar slants (Difco, Detroit, MI) and stored at 4 °C. The spores and/or mycelia were aseptically transferred from the agar slant to potato dextrose agar plates and allowed to grow for at least 48 h. The contents of two plates were then transferred to a sterile blender cup containing ~90 mL of sterile physiological saline solution and homogenized for 1 min. Approximately 5 mL of the homogenate was used to inoculate 125-mL Erlenmeyer flasks containing 30 mL of Sabouraud dextrose broth (SAB). The cultures were incubated for 48 h at 25 °C on a rotary shaker operating at 150 rpm, after which time 5 mg of triprolidine hydrochloride monohydrate dissolved in 500 μL of sterile water were added. The incubation of a fungal culture without triprolidine added, and a flask containing triprolidine, SAB (30 mL), and no microorganism, served as controls. After incubation, each flask was extracted and analyzed by high-performance liquid chromatography (HPLC) as described below.

Triprolidine Metabolite Extraction, Isolation, and Chemical Identification—Cultures of *C. elegans* ATCC 9245 were prepared and dosed with 5 mg of triprolidine hydrochloride monohydrate. After timed periods of incubation, the flask contents were transferred into 160-mL screw-cap culture tubes and 50 mL of methanol were added. The tubes were sealed with teflon-lined caps and vigorously shaken by hand for 1 min, and the fungal cells were pelleted by centrifugation at 2,000 rpm for 10 min. The supernatant was decanted to a 500-mL separatory funnel and the cell pellet was washed twice with 50 mL of methanol. About 100 mL of water were added to the combined separatory funnel contents and the aqueous:methanol mixture was extracted with five 100-mL volumes of methylene chloride. The organic extracts were combined by filtering through a plug of anhydrous sodium sulfate into a round-bottomed flask and evaporated to dryness under reduced pressure (water aspirator) at 40 °C. The residue was dissolved in the HPLC mobile phase and analyzed for unmetabolized triprolidine and triprolidine metabolites by HPLC.

The HPLC parameters have been previously reported.[8] Briefly, a conventional cyano-bonded phase column was eluted with a mobile phase of aqueous phosphate buffer (0.01 M KH_2PO_4, 0.02 M trimethylamine, pH 7.0) and acetonitrile (60:40; v/v). The HPLC hardware

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consisted of a model 100A high-pressure pump (Beckman Instruments/Altex Scientific Operations, Berkeley, CA) operated at 2 mL/min, a model 160 absorbance detector (Beckman) set at 254 nm, and a model 4100 computing integrator (Spectra-Physics, Santa Clara, CA). The samples were injected into the system using either a model 7125 septumless syringe loading injector (Rheodyne, Cotati, CA) or a WISP 710B autoinjector (Waters Chromatography Division, Milford, MA).

In order to isolate the major metabolites and unmetabolized triprolidine in quantities sufficient for structure identification and biological testing, the extract was subjected to repeated HPLC separations, and the UV-absorbing compounds with similar HPLC retention times were pooled and evaporated to a dry residue under reduced pressure (water aspirator) at 50 °C. The residue from each pooled fraction was dissolved in 5 mL of water and further purified using a C_{18} Sep-Pak cartridge (Waters). Each sample was loaded onto the C_{18} cartridge in a dropwise manner, using a 10-mL luer-lock glass syringe, and washed with two 5-mL aliquots of water. The compounds were eluted from the cartridge in 10 mL of methanol, evaporated to a dry residue, and stored at 4 °C prior to structure analysis.

To identify the isolated compounds, each sample was dissolved in methylene chloride-d_2 or methanol and the structure was determined using proton nuclear magnetic resonance (^{1}H-NMR) and ammonia and methane desorption chemical ionization mass spectral (DCIMS) techniques. The ^{1}H-NMR measurements were carried out at 270 MHz on a WH 270 spectrometer (Bruker Instruments, Inc., Billerica, MA). The data were acquired under the following conditions: data size, 16,000; sweep width, 3500 Hz; filter width, 4400 Hz; temperature, 300 °K; flip angle, 80°. All DCIMS measurements were performed using a Finnigan MAT 4023 mass spectrometer and a direct-exposure probe. The instrumentation parameters used for the DCIMS analyses have been described in detail elsewhere.[8]

Oxygen-18 Incorporation Experiments—Cells of *C. elegans* ATCC 9245 were grown in Erlenmeyer flasks as previously described. After 48 h of incubation, the SAB medium was decanted from each flask and the cells were washed twice with 50-mL aliquots of sterile physiological saline solution. The cells from three flasks were transferred to a 500-mL Erlenmeyer flask and suspended in 100 mL of sterile phosphate buffer solution (pH 7.2). Five milligrams of triprolidine hydrochloride monohydrate were added and the flask was sealed with a rubber septum. The flask was repeatedly deaerated using a vacuum pump and flushed with argon to remove the $^{16}O_2$ present. Finally, $^{18}O_2$ (99.8 atom%, Mound Facility, Monsanto Corp., Miamisburg, OH) was introduced into the flask with a cannula. The initial atmospheric isotopic composition of the closed system was immediately determined and found to be 98 atom% ^{18}O. After 72 h of incubation, the isotopic composition was determined to be ~90 atom% ^{18}O, demonstrating the integrity of the closed system. These ^{18}O:^{16}O ratios were determined using a Finnigan MAT 1015 quadrupole mass spectrometer. The ion source was operated with an electron energy of 70 eV. The gas samples were removed from the flask using a 1.0-mL gas-tight syringe and rapidly injected into the reservoir probe which was inserted in the mass spectrometer. During these analyses, the sample pressure was adjusted via a Granville-Phillips variable leak valve until full scale deflection of the oxygen isotopes cluster was obtained (~5×10^{-6} Torr analyzer pressure). The hydroxymethyl triprolidine produced after 72 h of incubation was isolated by HPLC as previously described. Identification of the metabolite was confirmed from its electron impact (EI) mass spectrum which was obtained using a Finnigan MAT 4023 mass spectrometer, operating at 70 eV ionizing voltage, with a direct exposure probe for sample introduction. The ratio of ^{18}O and ^{16}O incorporation in the metabolite was determined from the relative intensities of the ^{18}O and ^{16}O species observed at *m/z* 296 and 294, respectively.

Kinetics of Triprolidine Metabolism by *C. elegans* ATCC 9245—Kinetic experiments were conducted by determining the amount of unmetabolized triprolidine recovered from growing cultures of *C. elegans* ATCC 9245 at various timed intervals. A series of flasks were prepared and dosed with 5 mg of triprolidine hydrochloride monohydrate. At 24, 72, 120, 196, 216, and 240 h, duplicate triprolidine-dosed fungal cultures were removed from incubation and prepared for HPLC analysis. A sterile sample which contained only SAB and triprolidine (no fungus) served as a quantitative standard and was used to determine the amount of triprolidine and to estimate the amount of the hydroxymethyl metabolite that was present in the dosed fungal cultures. The quantitation was performed by an external standard method using integrated peak area measurements which were generated by a model SP-4100 (Spectra-Physics) computing integrator.

Mutagenicity Assays—Mutagenicity was determined using *Salmonella typhimurium* tester strains TA97, TA98, TA100, and TA104, essentially as described by Maron and Ames.[16] The assays were conducted in the absence of exogenous activation and in the presence of 50 μL per plate of a hepatic post-mitochondrial supernatant (S9) fraction prepared from male Sprague-Dawley rats pretreated with Aroclor 1254. All assays were performed in triplicate and revertant colonies were counted using a Biotran III (New Brunswick Scientific, Edison, NJ).

Results and Discussion

Preliminary Studies—Triprolidine hydrochloride monohydrate (5 mg) was dissolved in sterile physiological saline and added to 48-h cultures of *C. elegans* ATCC 9245. At timed intervals of incubation, 10-μL aliquots of culture medium were removed and injected directly into the HPLC. The chromatographic profiles obtained were used to monitor the metabolism of the antihistamine. After 192 h of incubation, the dosed cultures and control flasks were extracted with methylene chloride and the extracts were analyzed by HPLC. Figure 1 shows the HPLC elution profiles of the organic extractable components of a 192-h SAB:triprolidine control (Figure 1A) and dosed *C. elegans* culture (Figure 1B). Figure 1B shows two major compounds eluting at 8.3 (Peak I) and 12.0 min (peak II). Compounds 1 and 2 were collected and concentrated in quantities sufficient to permit structure identification and biological testing. Many of the minor compounds shown in Figure 1B, that eluted between 0 and 4 min, were detected in the control sample (Figure 1A). This indicates that these minor compounds may not be triprolidine metabolites and were most probably derived from the culture medium. The minor compounds that were not detected in the control sample were not collected in sufficient quantity to allow structural identification.

Isolation and Identification of Compounds 1 and 2—The mass spectral analyses of 1 and 2 were performed using DCIMS because this technique has been shown to be useful

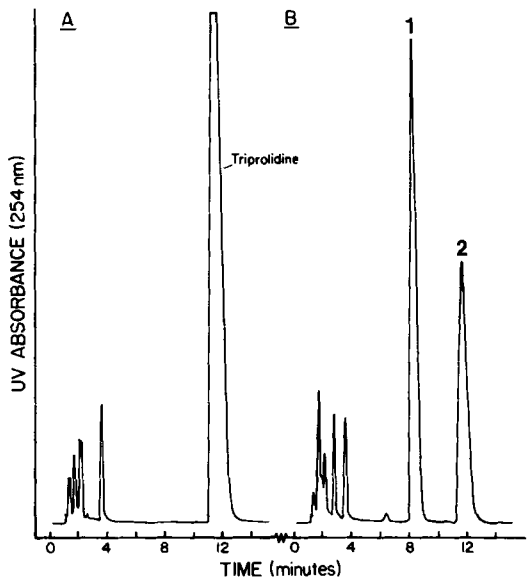

Figure 1—*Typical HPLC-UV elution profiles obtained from* (A) *the 192-h SAB:triprolidine control sample and* (B) *the 192-h triprolidine-dosed* C. elegans *ATCC 9245 sample. The major peaks labeled* 1 *and* 2 *were identified as hydroxymethyl triprolidine and triprolidine, respectively.*

for the analysis of similar antihistamines and their metabolites.[8,17-20]

The methane DCI mass spectrum of the authentic triprolidine (as the free base) yielded a base peak at *m/z* 279, corresponding to the $[M + H]^+$ ion. Other peaks observed included a small peak at *m/z* 307, corresponding to the $[M + C_2H_5]^+$ ion, and a large peak at *m/z* 208, which is attributed to the $[M + H - NH{-}C_4H_8]^+$ fragment ion (i.e., loss of the five-membered ring). The ammonia DCI mass spectrum for triprolidine showed essentially only the base peak at *m/z* 279. The 270 MHz ^{1}H-NMR spectrum of the authentic triprolidine standard was determined and the assigned chemical shifts and coupling constants are as follows: (Methylene chloride-d_2):1.71 (4H, m, 3″ − CH_2), 2.39 (3H, s, 4′ − CH_3), 2.46 (4H, m, 2″ − CH_2), 3.14 (2H, d, $J_{\alpha,\beta}$ = 6.7 Hz, αCH_2), 6.88 (1H, t, βCH), 7.00 (1H, d, $J_{3,4}$ = 7.8 Hz, H_3), 7.07 (2H, ad, $H_{3'}$), 7.10 (1H, dd, $J_{4,5}$ = 7.8 Hz, $J_{5,6}$ = 4.7 Hz, H_5), 7.21 (2H, ad, $H_{2'}$), 7.53 (1H, dd, H_4), and 8.53 (1H, d, H_6) ppm.

Compound 2, eluting at 12.0 min, was identified as unmetabolized triprolidine since its methane DCI mass spectrum, ammonia DCI mass spectrum, and ^{1}H-NMR spectrum were consistent with the authentic triprolidine spectroscopic data described above.

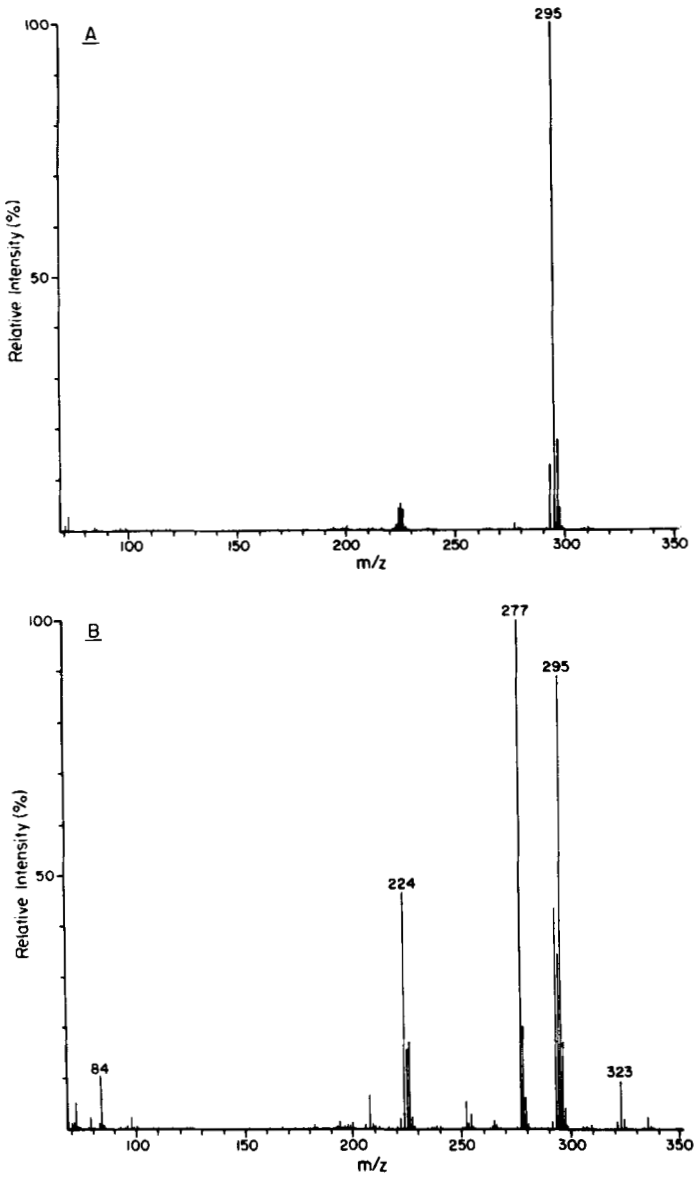

Figure 2—*The* (A) *ammonia and* (B) *methane desorption chemical ionization mass spectra (DCIMS) of* 1 *(hydroxymethyl triprolidine).*

Compound 1 (HPLC retention time 8.3 min) was identified as hydroxymethyl triprolidine (2-[1-(4-hydroxymethylphenyl)-3-(1-pyrrolidinyl-1-propenyl)] pyridine) based on its DCIMS and ^{1}H-NMR analysis. Figure 2A shows the ammonia DCI mass spectrum for this metabolite. The base peak at *m/z* 295 is presumably the $[M + H]^+$ ion. The methane DCI mass spectrum of the metabolite is shown in Figure 2B. This spectrum includes a large peak at *m/z* 295 and a smaller peak at *m/z* 323, corresponding to the $[M + H]^+$ and the $[M + C_2H_5]^+$ ions, respectively, and confirming that the metabolite has a molecular weight of 294. The base peak in the methane DCI mass spectrum was at *m/z* 277, corresponding to a loss of water (i.e., the $[M + H - H_2O]^+$ ion). The other major ion in the spectrum at *m/z* 244 is presumably due to cleavage of the five-membered ring (i.e., the $[M + H - NH{-}C_4H_8]^+$ ion). This metabolite was also analyzed by EIMS (vide infra), and the results were found to be in agreement with the EIMS data reported previously for hydroxymethyl triprolidine.[14] The ^{1}H-NMR data of 1 were measured and the assigned chemical shifts and coupling constants are as follows: (methylene chloride-d_2):1.72 (4H, m, 3″ − CH_2), 2.47 (4H, m, 2″ − CH_2), 3.14 (2H, d, $J_{\alpha,\beta}$ = 7.0 Hz, αCH_2), 4.71 (2H, s, 4′ − CH_2), 6.88 (1H, t, βCH), 7.02 (1H, d, $J_{3,4}$ = 7.8 Hz, H_3), 7.13 (1H, $J_{4,5}$ = 7.8 Hz, $J_{5,6}$ = 4.7 Hz, H_5), 7.17 (2H, ad, $H_{3'}$), 7.40 (2H, ad, $H_{2'}$), 7.55 (1H, t, H_4), and 8.53 (1H, d, H_6) ppm. We compared the ^{1}H-NMR spectra of triprolidine (Figure 3A) with that of 1 (Figure 3B). These data are consistent with oxidation of the methyl group of triprolidine since the only significant difference between these two spectra is the presence of a two-proton singlet at 4.71 ppm and the absence of the methyl resonance at 2.39 ppm. These ^{1}H-NMR results for 1 are in agreement with previous ^{1}H-NMR data reported for this compound.[14]

The hydroxymethyl derivative has been identified as a major metabolite produced from the in vitro incubation of triprolidine with guinea pig microsomes.[14] This compound is reported to undergo further metabolism by the soluble liver enzyme fraction to the carboxylic acid derivative which has been detected in the urine of dosed animals. Experiments with *C. elegans* ATCC 9245 were performed to determine whether or not secondary fungal oxidation of the hydroxymethyl metabolite to the acid derivative occurred. Triplicate 48-h cultures were dosed with ~1 mg each of the hydroxymethyl derivative. To serve as a control and quantitation standard, a flask was prepared containing only SAB and hydroxymethyl triprolidine. After 192 h of incubation, the samples were analyzed by HPLC. Comparison of HPLC peak areas indicated essentially 100% recoveries of the unchanged hydroxymethyl metabolite in each case (data not shown). These results suggest that *C. elegans* does not form appreciable amounts of the carboxylic acid derivative of triprolidine through oxidation of the hydroxymethyl derivative.

Oxygen-18 Incorporation into Hydroxymethyl Triprolidine—Cells of *C. elegans* were incubated with triprolidine in the presence of 98 atom% ^{18}O oxygen for ~72 h. The hydroxymethyl triprolidine formed was isolated and the percent incorporation of ^{18}O was determined by mass spectrometry. Figure 4 shows the EI mass spectra of the hydroxymethyl triprolidine formed in the presence of $^{16}O_2$ and $^{18}O_2$ (inset). These spectra show an increase in the $[M + 2]^+$ peak (*m/z* 296) for the hydroxymethyl triprolidine formed under $^{18}O_2$ conditions, and indicate a >90% incorporation of ^{18}O into the metabolite. These results were further confirmed by ammonia DCIMS analysis of the ^{18}O-labeled metabolite which demonstrated a base peak at *m/z* 297 corresponding to the $[M + H]^+$ ion for this compound (data not shown). The oxygen-18 incorporation experiment demonstrates that mo-

lecular oxygen is necessary for enzymatic attack at the methyl group of triprolidine by *C. elegans* and, by analogy with previous studies,[21] the reaction is most likely being catalyzed by cytochrome P-450 mono-oxygenase.

Kinetics of Triprolidine Transformation by *C. elegans* ATCC 9245—The time course for the formation of the hydroxymethyl derivative and decrease of triprolidine was measured and the data are shown in Figure 5. After 24 h of incubation, 94.0% of the triprolidine added was recovered unchanged. Detection of the hydroxymethyl metabolite began after 72 h of incubation at ~4.4% and increased to 55% of the initial dose after 240 h. The amount of unmetabolized triprolidine recovered after 240 h was ~23.0%. The remaining 22% of the initial dose was assumed to be either adsorbed to the fungal cells and/or unidentified water-soluble triprolidine metabolites remaining in the extracted aqueous media.

Mutagenicity of Triprolidine and Its Hydroxymethyl Metabolite in *Salmonella typhimurium*—The mutagenic activity of triprolidine and its hydroxymethyl analogue was measured using *Salmonella typhimurium* strains TA97, TA98, TA100, and TA104. These assays were performed in the absence and presence of hepatic S9 prepared from rats treated with Aroclor 1254. Benzo[*a*]pyrene in the presence of S9 activation and methylmethanesulfonate without the S9 activation were assayed as positive controls. The triprolidine tested was obtained from the commercial source described in the *Experimental Section* and was assayed at concentrations up to 1.0 mg/plate. The hydroxymethyl triprolidine metabolite was obtained through fungal transformation and was assayed at concentrations up to 200 μg/plate. The data shown

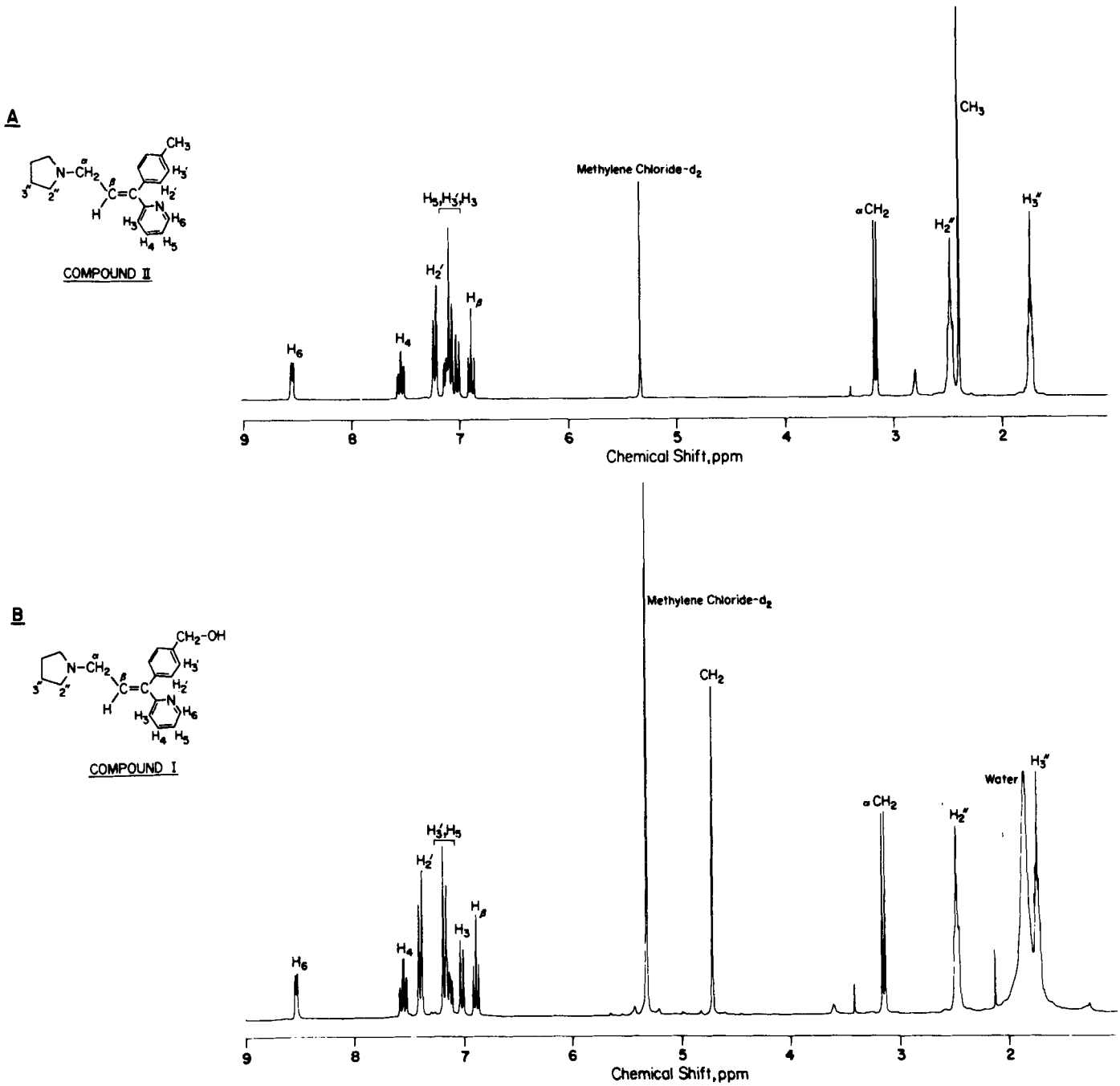

Figure 3—*Proton NMR spectra (270 MHz) with assignments of* (A) *authentic triprolidine and* (B) *compound* 1 *(hydroxymethyl triprolidine) isolated from cultures of* C. elegans *ATCC 9245 grown in the presence of triprolidine.*

in Table I indicate that at the concentrations tested, neither compound demonstrates appreciable mutagenic activity in any of the *Salmonella* tester strains. Both compounds were assayed on at least two different occasions with similar results. These results indicate that the metabolism of triprolidine to the hydroxymethyl analogue occurs through pathways which do not result in mutagenic activation.

Conclusions

Microbial transformation methodologies have been used to synthesize bioactive compounds of toxicological interest and have complemented mammalian metabolism research.[6-8,21,22] The filamentous fungus *Cunninghamella elegans* has both phase I and phase II enzyme activities and metabolizes a wide variety of organic compounds, including substituted and unsubstituted polycyclic aromatic hydrocarbons,[21] triarylphosphate esters,[23] and pharmaceutical drugs including antihistamines.[7,8] In this paper, we describe an alternative to organic synthesis for production of mg quantities of a known mammalian metabolite of triprolidine through microbial transformation methodologies employing *C. elegans*. This alternative method is particularly useful in the case of triprolidine since there appears to be no simple and direct synthetic route from triprolidine to the hydroxymethyl derivative due to the reactivity of the vinyl carbons.

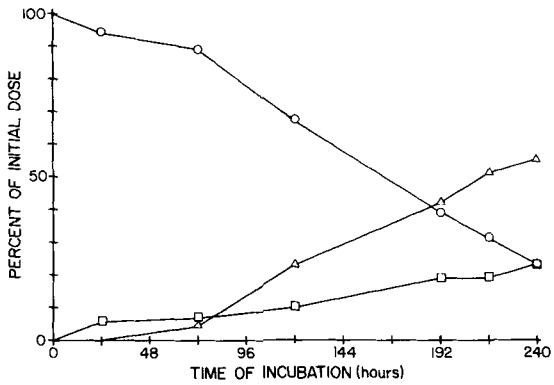

Figure 5—*Kinetics data showing the conversion of triprolidine by C. elegans ATCC 9245 with time. Key: (○) the percent of the initial triprolidine dose recovered unchanged; (△) the percent of the initial dose determined as hydroxymethyl triprolidine; (□) the percent of the initial dose that was not identified as triprolidine or hydroxymethyl triprolidine. The initial dose of antihistamine added to ~30 mL of culture was 5 mg of triprolidine hydrochloride.*

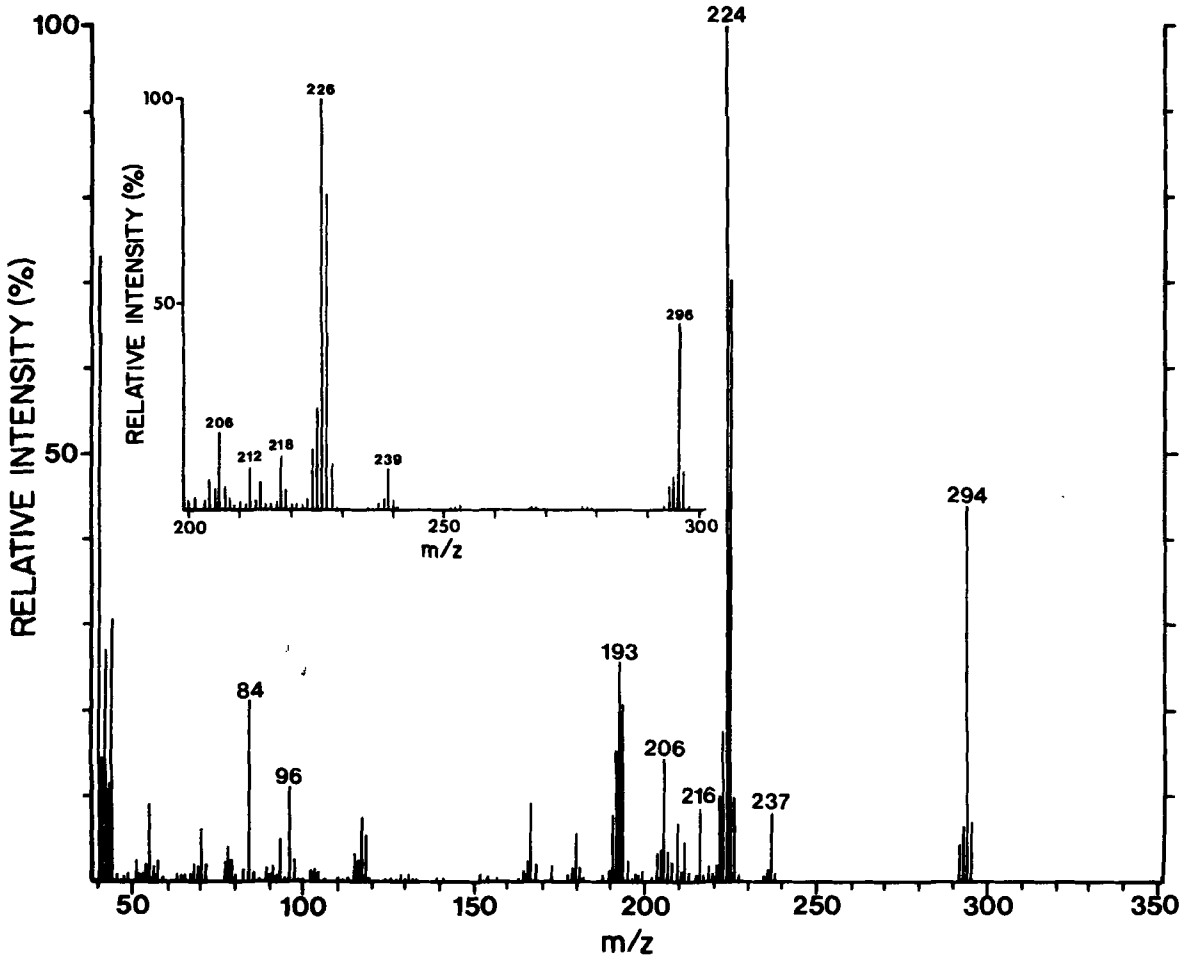

Figure 4—*Electron impact mass spectra of hydroxymethyl triprolidine formed by* C. elegans *in the presence of* $^{16}O_2$ *and* $^{18}O_2$ *(inset).*

Table I—Mutagenicity of Triprolidine and Its Hydroxymethyl Metabolite Formed by *C. elegans* ATCC 9245

Compound	μg/plate	Revertants/Plate[a]							
		TA 97		TA 98		TA 100		TA 104	
		+S9	−S9	+S9	−S9	+S9	−S9	+S9	−S9
DMSO (solvent)	100 μL	152 ± 2	90 ± 29	14 ± 3	13 ± 4	186 ± 17	176 ± 22	544 ± 15	458 ± 75
Methyl methanesulfonate (MMS)	1 μL	—	209 ± 18	—	32 ± 6	—	1203 ± 60	—	1549 ± 36
Benzo(*a*)pyrene	5	1353 ± 37	—	373 ± 20	—	1342 ± 339	—	1371 ± 160	—
Triprolidine	15	174 ± 14	120 ± 32	16 ± 7	16 ± 2	198 ± 23	194 ± 18	457 ± 21	419 ± 58
	50	154 ± 11	137 ± 14	18 ± 7	14 ± 4	202 ± 21	193 ± 16	449 ± 28	410 ± 27
	100	167 ± 16	158 ± 16	21 ± 1	13 ± 5	184 ± 29	173 ± 14	472 ± 42	409 ± 26
	500	165 ± 17	101 ± 19	22 ± 4	15 ± 4	166 ± 7	151 ± 5	580 ± 63	480 ± 44
	1000	217 ± 16	155 ± 13	17 ± 4	12 ± 2	203 ± 26	171 ± 19	498 ± 15	353 ± 72
DMSO	100 μL	188 ± 8	130 ± 4	25 ± 6	15 ± 5	170 ± 13	157 ± 8	448 ± 73	412 ± 64
Benzo(*a*)pyrene	5	1450 ± 96	—	684 ± 98	—	920 ± 100	—	1282 ± 13	—
MMS	1 μL	—	193 ± 31	—	29 ± 2	—	1360 ± 94	—	1858 ± 85
Hydroxymethyl triprolidine	15	181 ± 20	132 ± 27	25 ± 2	26 ± 7	163 ± 8	152 ± 1	458 ± 28	380 ± 33
	50	194 ± 25	119 ± 32	25 ± 2	18 ± 6	177 ± 9	150 ± 12	400 ± 25	359 ± 57
	100	177 ± 23	145 ± 41	26 ± 2	23 ± 3	190 ± 24	139 ± 26	370 ± 15	313 ± 24
	200	171 ± 30	143 ± 9	22 ± 5	16 ± 1	124 ± 23	146 ± 15	287 ± 29	357 ± 54

[a] Values are the mean number of revertants and standard deviations of triplicate assays.

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Acknowledgments

We thank James P. Freeman for the mass spectral analyses of the $^{18}O_2$ atmosphere in the oxygen-18 incorporation experiment, Janice Thornton-Manning for her assistance with the mutagenicity assays, and Daniel Nestorick for the drawings.