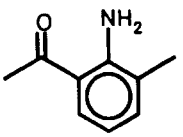
elution sequence was that presented above and in Table I in all cases. This evidence confirms that, in all of the examples reported by Kimmel et al. (1986) and in the current study, the GC peak that should be assigned as the nitroso compound elutes earlier than the corresponding aniline. This elution sequence is also consistent with the relative position of 2,6-diethylnitrobenzene (11) (Table I), since 11 is expected to behave similarly to undecomposed 1 on the GC but elute somewhat later due to its greater molecular weight.

On the basis of the relative peak positions reported by Kimmel et al. (1986), it appears that they may have misassigned the peak caused by 12, which has been positively identified in the present study by comparison with an authentic sample as described above, as the nitroso derivative 1. Possibly the analogous compound 22 may have also been misassigned as 8. Such an error would be easy to make without a thorough study of the decomposition process since these pairs of compounds are isomers with very similar mass spectra except for the fragments resulting from loss of CH_3CO from the molecular ions.



22

CONCLUSIONS

In the present study, 2,6-diethylnitrosobenzene (1) has been shown to have physical properties and an unexpected instability in solution, which complicate its analysis. Methods that rely on GC separations are inappropriate, especially because the extensive decomposition of 1 during the analysis produces substances that are isomeric with 1, resulting in possible misinterpretations even when GC/MS is used. In contrast, HPLC/MS analysis of freshly prepared solutions of 1 produce excellent results and can be accomplished with only minor peak broadening due to the equilibrium between the monomeric and dimeric forms 1 and 4. The application of this methodology to metabolic samples that may contain 1 is discussed in the accompanying paper by Feng and Wratten (1987). The instability of 1 in solution is also likely to complicate the interpretation of results from biological tests requiring days to complete, such as bacterial mutagenicity testing. Positive results from such testing may arise from the decomposition products 14–16 rather than from 1 itself. Similarly, studies with 2-ethyl-6-methylnitrosobenzene (8) are also subject to uncertainties due to decomposition, which are less severe than for 1, while 2,6-dimethylnitrosobenzene (6) is almost entirely exempt from these phenomena. The extent to which studies of other alkylated nitrosobenzene derivatives of potential toxicological or metabolic interest are plagued by such concerns remains unknown.

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In Vitro Oxidation of 2,6-Diethylaniline by Rat Liver Microsomal Enzymes

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Incubation of 2,6-diethylaniline (DEA) with NADPH-fortified rat liver microsomal enzymes produced 4-amino-3,5-diethylphenol (ADEP) as the major product of oxidation. ADEP was shown to undergo further oxidation to 3,5-diethylbenzoquinone 4-imine (DEBQI), which was isolated as a minor metabolite during DEA oxidation. Metabolites resulting from N-oxidation or alkyl hydroxylations were not observed.

N-Oxidation of arylamines is generally believed to result in the formation of reactive metabolic intermediates that are responsible for some of their toxic effects (Weisburger and Weisburger, 1973). Kimmel et al. (1986) recently reported the in vitro and in vivo metabolic conversion of several 2,6-dialkylchloroacetanilide herbicides to the corresponding 2,6-dialkylanilines by rats. The anilines were in turn reported to be converted to the corresponding 2,6-dialkylnitrosobenzenes, which were shown to be direct-acting mutagens by the Ames assay. These studies

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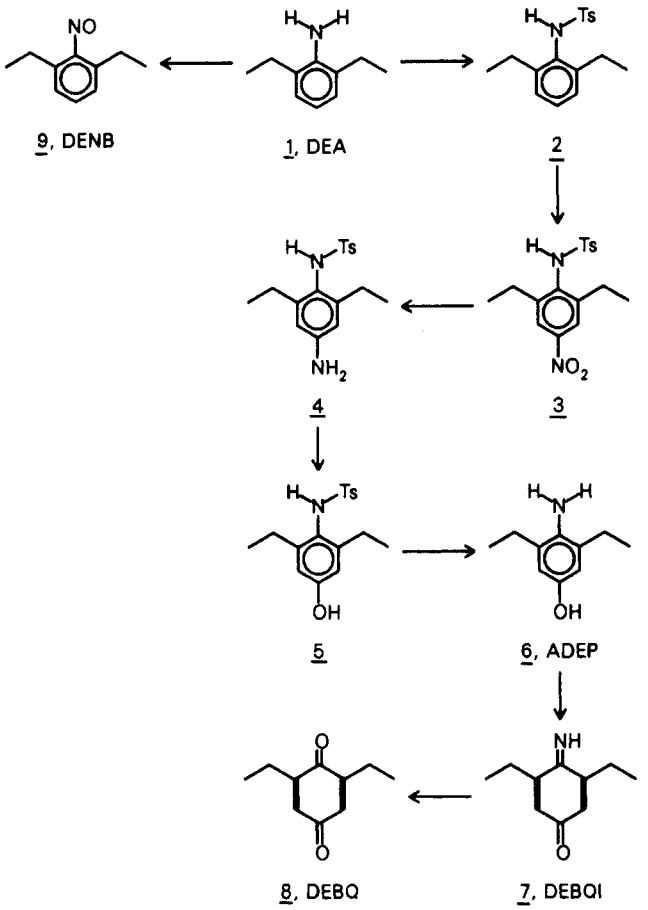

Figure 1. Pathways of chemical synthesis of authentic standards (Ts = *p*-toluenesulfonyl).

were conducted with use of nonradiolabeled substrates, and the nitrosobenzene products were detected by selected ion monitoring with gas chromatography/mass spectroscopy (GC/MS/SIM); the formation of other metabolites was not studied.

We have been studying the metabolism of chloroacetanilide herbicides. The reported biotransformation of alachlor [2-chloro-*N*-(methoxymethyl)-*N*-(2,6-diethylphenyl)acetamide] by rat liver enzymes to produce 2,6-diethylaniline (DEA, 1; Figure 1) and then 2,6-diethylnitrosobenzene (DENB, 9) (Kimmel et al., 1986) contradicted the results from our DEA metabolism studies and prompted us to conduct further investigations. The identification of metabolites from the in vitro metabolism of DEA by rat liver microsomal enzymes is presented in this report.

EXPERIMENTAL SECTION

Chemicals. The ^{14}C-labeled [U-phenyl]DEA (13.5 mCi/mmol) was obtained from New England Nuclear, Inc. The chemical and radiochemical purity of [^{14}C]DEA was measured by HPLC to be greater than 99%. DEA, anisole, and *m*-chloroperoxybenzoic acid (MCPBA) were purchased from Aldrich Chemical Co., and reduced nicotinamide adenine dinucleotide phosphate (NADPH) was obtained from Sigma Chemical Co.

Liver Homogenate Preparation. Livers were obtained from male Long Evans rats (average body weight 250 g). The animals were sacrificed by cervical dislocation, and their livers were removed immediately. The livers from all the animals were combined, rinsed with ice-cold buffer (0.01 M phosphate, pH 7.4, 1.15% KCl), and homogenized with the same buffer with a Teflon pestle and 3 mL of buffer/g of tissue. The homogenate was centrifuged at 9000*g* for 30 min, the supernatant was filtered through cheesecloth to remove lipids, and the resultant preparation was the S9 fraction. Further centrifugation of the S9 fraction at 100000*g* for 1 h produced a pellet, which was resuspended to produce the microsomal fraction. All procedures were conducted at 0 °C, and the enzyme fractions were stored at −20 °C. The protein concentrations were measured by the Coomassie blue dye-binding assay (Bradford, 1976).

Liver Microsomal Incubations. In a final volume of 1 mL, 1–40 μg (0.007–0.27 μmol) of [^{14}C]DEA (1 mCi/mmol, in <10 μL of ethanol) was incubated with the liver microsomal fraction (5 mg of protein) in the presence of reduced NADPH (2 μmol) and pH 7.4 phosphate buffer (0.2 mM) at 37 °C. Control incubations were conducted in the absence of NADPH and with the heat-denatured enzymes. The reaction mixtures were sampled (100 μL) at various time intervals, and the proteins were precipitated with 50 μL of ice-cold methanol. Following centrifugation, the resulting supernatant was analyzed by HPLC.

Metabolite Isolation and Purification. The incubation of DEA with liver microsomal enzymes produced one major and one minor metabolite as evidenced by HPLC analysis. The methanol precipitation of the proteins recovered 105% of the radioactivity in the supernatant. Hexane extraction of the crude incubation mixture, as described by Kimmel et al. (1986), produced only a 70% recovery. Therefore, the metabolites in the deproteinated solution were extracted into methylene chloride prior to mass spectral analyses. DEA was very volatile, and minor loss of radioactivity was observed during rotary evaporation or nitrogen evaporation of the methylene chloride extract. The major metabolite in the incubation mixture was also purified by multiple HPLC injections during which the effluent corresponding to the radioactivity peak was collected.

Instrumentation. The HPLC analyses were conducted on a system assembled from components manufactured by Waters Associates (two Model 6000A pumps, a Model 680 gradient controller, a Model 440 UV detector). The analyses employed a Waters μBondapak C_{18} column (3.9 mm × 30 cm) with a linear gradient of 20–100% acetonitrile in 5 mM acetate buffer (pH 4.6) in 15 min at a flow rate of 1 mL/min. The HPLC effluent was sequentially detected by a UV (254-nm) monitor and by a radioactivity flow monitor (Packard RAM 7500) equipped with a solid scintillant cell. GC/MS analyses in the electron impact (EI) and chemical ionization (CI, isobutane) modes were conducted on a Finnigan MAT 4535 quadrupole system using a cross-linked methyl silicone column (10 m × 0.32 mm) from Hewlett-Packard (isothermal 50 °C for 1 min, 20 °C/min to 130 °C, and 10 °C/min to 250 °C; injector 150–250 °C). Scanning high-resolution GC/MS analyses in the EI mode (25 m × 0.32 mm DB-5 column from J & W Scientific; isothermal 100 °C for 1 min, 10 °C/min to 250 °C; injector 200–250 °C) and fast atom bombardment (FAB) analyses in glycerol matrices were conducted on a VG ZAB-HF instrument. HPLC/MS analyses with filament ionization were conducted on a Waters HPLC system coupled to the Finnigan MAT 4535 instrument with a thermospray interface (Vestec Inc.). The HPLC gradient employed the same column as above with a linear gradient of 25–75% acetonitrile in water in 20 min at a flow rate of 1 mL/min. NMR spectra were recorded on either a Varian EM60 or a Varian XL-300 spectrometer in $CDCl_3$, with proton chemical shifts assigned relative to $CHCl_3$ at 7.26 ppm.

4-Amino-3,5-diethylphenol (ADEP, 6). The synthesis of ADEP was accomplished from DEA (1) in a five-step sequence (Figure 1). 4-Methyl-*N*-(2,6-diethylphenyl)-benzenesulfonamide (2), prepared by the treatment of DEA with *p*-toluenesulfonyl chloride (Bavin and Scott, 1958), was dissolved in acetic acid (50 mL) containing sodium nitrite (2.4 g, 35 mmol), and the mixture was stirred at 80 °C. Nitric acid (90%, 13.5 mL) was dissolved in acetic acid (20 mL) and added dropwise over a 10-min period. The resulting red fuming mixture was heated to approximately 95 °C for a additional 45 min, at which time it was cooled to 25 °C and poured onto ice. The mixture was extracted with methylene chloride (3 × 50 mL). The combined organic layers were dried (Na_2SO_4), evaporated, and crystallized from hot methanol to yield 3.5 g (30% theoretical) of 4-methyl-*N*-(2,6-diethyl-4-nitrophenyl)-benzenesulfonamide (3), as yellow crystals: NMR δ 8.18 (s, 2 H), 8.11 (d, 2 H), 7.52 (d, 2 H), 2.67 (q, 4 H), 2.54 (s, 3 H), 1.24 (t, 6 H); CI mass spectrum, *m/z* 349 (MH^+), 195, 157; EI high-resolution mass measurement, observed 348.1170, $C_{17}H_{20}N_2O_4S$ requires 348.1144.

The sulfonamide 3 (3.7 g, 10.6 mmol) was dissolved in ethanol–THF (1:1; 100 mL) containing 5% Pd on carbon (800 mg) and the resultant mixture agitated under hydrogen (50 psi) for 5 h in a Parr apparatus. The resulting solution was filtered and evaporated to yield a viscous yellow oil that was crystallized as the hemisulfate salt from hot 0.5 N aqueous H_2SO_4. Two crops of crystals were collected to yield 2.9 g (65% of theoretical). This salt was converted to the free amine, 4-methyl-*N*-(4-amino-2,6-diethylphenyl)benzenesulfonamide (4), in quantitative yield by partitioning it between methylene chloride and 1.0 N NaOH: NMR δ 7.62 (d, 2 H), 7.18 (d, 2 H), 6.34 (s, 2 H), 2.40 (S, 3 H), 2.34 (q, 4 H), 0.98 (t, 6 H); CI mass spectrum, *m/z* 319 (MH^+), 165, 157; EI high-resolution mass measurement, observed 318.1406, $C_{17}H_{22}N_2O_2S$ requires 318.1402.

The hemisulfate salt of amine 4 (2.0 g, 4.8 mmol) was stirred in acetonitrile–ethanol (1:1; 70 mL) containing H_2SO_4 (1.0 g, 10 mmol) and water (2 mL) at 3 °C. A solution of *tert*-butyl nitrite (1.6 mL, 16.7 mmol, 3.5 equiv) in acetonitrile (35 mL) was added dropwise over a 10-min period. The mixture was warmed to 10 °C, stirred for 30 min, and then concentrated on the rotary evaporator to approximately 25 mL while a 10 °C temperature was maintained. The product was diluted with dioxane–0.2 N aqueous H_2SO_4 (1:1; 70 mL) and added dropwise to 0.2 N H_2SO_4 (250 mL), preheated to 70 °C. The resulting mixture was heated briefly to reflux, and evolution of nitrogen was noted. It was cooled to room temperature and extracted with ethyl ether (3 × 75 mL). The combined, dried organic layers were evaporated to leave 4-methyl-*N*-(2,6-diethyl-4-hydroxyphenyl)benzenesulfonamide (5), as a viscous oil that slowly solidified: 1.5 g (95% of theoretical); NMR δ 7.60 (d, 2 H), 7.22 (d, 2 H), 6.56 (s, 2 H), 2.41 (s, 3 H), 2.34 (q, 4 H), 0.98 (t, 6 H); CI mass spectrum, *m/z* 320 (MH^+), 166, 157; high-resolution mass measurement, observed 319.1242, $C_{17}H_{21}NO_3S$ requires 319.1242.

Phenol 5 (78 mg, 0.24 mmol) and anisole (400 mg, 3.7 mmol) were combined in a Teflon vessel and the resultant mixture stirred at −78 °C while HF (approximately 5 mL) was condensed into the vessel. The mixture was warmed to 0 °C and stirred for 1 h, after which the HF was removed under vacuum. Methanol (10 mL) containing triethylamine (1 g) was used to dissolve the product, and evaporation left a thick oil that was dissolved in water (3 mL) and adjusted to pH 2. The insoluble oil was removed by extraction with methylene chloride (3 × 3 mL) and discarded, and the aqueous layer was adjusted to pH 6.5. Reextraction with methylene chloride (3 × 3 mL) produced a second organic extract that was combined, dried, and evaporated to leave ADEP (6) as tan crystals [37 mg (93% of theoretical)], which could be recrystallized from toluene–cyclohexane (1:1) to yield fine, slightly greenish needles: mp 105–107 °C with sublimation; NMR δ 6.52 (s, 2 H), 2.51 (q, 4 H), 1.26 (t, 6 H); EI mass spectrum, *m/z* 165 ($M^{\bullet+}$), 150; EI high-resolution mass measurement, observed 165.1122, $C_{10}H_{15}NO$ requires 165.1154.

3,5-Diethylbenzoquinone 4-Imine (DEBQI, 7). Small-scale synthesis of DEBQI was accomplished by oxidation of ADEP (6; 300 μg) in ethyl acetate (1 mL) with lead tetraacetate (2 mg). After 5 min of reaction, water was added and the products were partitioned into ethyl acetate. HPLC and GC analyses demonstrated the formation of one major and one minor product. The major product (95% of mixture) was identified by GC/MS analysis as DEBQI: EI mass spectrum, *m/z* 163 ($M^{\bullet+}$) 148, 135, 120; EI high-resolution mass measurement observed 163.1017, $C_{10}H_{13}NO$ requires 163.0997. The minor product, which accounted for less than 5% of the product, was identified by GC/MS analysis as 2,6-diethylbenzoquinone (DEBQ, 8): EI mass spectrum, *m/z* 164 ($M^{\bullet+}$), 149, 136, 121; EI high-resolution mass measurement observed 164.0856, $C_{10}H_{12}O_2$ requires 164.0837.

2,6-Diethylnitrosobenzene (DENB, 9). Oxidation of DEA with 2 equiv of MCPBA in acetonitrile produced the corresponding nitrosobenzene (Kimmel et al., 1986). DENB was extremely thermolabile; its chemical properties and decomposition products were studied by Wratten et al. (1987, preceding paper in this issue).

RESULTS AND DISCUSSION

The in vitro incubation of [^{14}C]DEA (40 μg, 1 mCi/mmol) with rat liver microsomal enzymes fortified with NADPH produced one major and one minor product as evidenced by HPLC analysis (Figure 2). The formation of these products was not observed in incubations without added NADPH or with heat-denatured enzymes, demonstrating that these reactions were enzyme catalyzed. After 0.5 h of incubation, the mixture was deproteinated with methanol, and liquid scintillation analysis of the supernatant showed 105% recovery of the total radioactivity. The major and the minor metabolites of DEA coeluted by HPLC with authentic standards of ADEP and DEBQI, respectively. After 0.5 h of incubation the percent distribution of radioactivities for ADEP, DEBQI, and unreacted DEA were 14%, 3%, and 83%, respectively. Longer incubation periods produced limited further conversion of DEA. However, if either the substrate concentration was reduced or the enzyme concentration was increased, then complete conversion of DEA to ADEP and DEBQI was observed.

The deproteinated reaction mixture was extracted with methylene chloride, and the extract was analyzed by GC/MS in both CI and EI modes. The CI mass spectral analysis confirmed the presence of DEA (*m/z* 150, MH^+, 100% relative intensity), ADEP (*m/z* 166, MH^+, 100%), and DEBQI (*m/z* 164, MH^+, 100%). The CI mass spectrum of DEBQI also showed ions at *m/z* 166 (25%), which resulted from the reduction of DEBQI to ADEP. The EI mass spectrum of DEA produced molecular ions at *m/z* 149 (65%) and fragment ions at *m/z* 134 ($M^{\bullet+}$ − 15, 100%) and 119 $M^{\bullet+}$ − 30, 20%). Similar fragment ions were observed in the EI mass spectrum of ADEP (Figure 3), with ions at *m/z* 165 ($M^{\bullet+}$ 95%), 150 ($M^{\bullet+}$ − 15, 100%), and 135 ($M^{\bullet+}$ − 30, 20%). Scanning high-resolution mass spectral

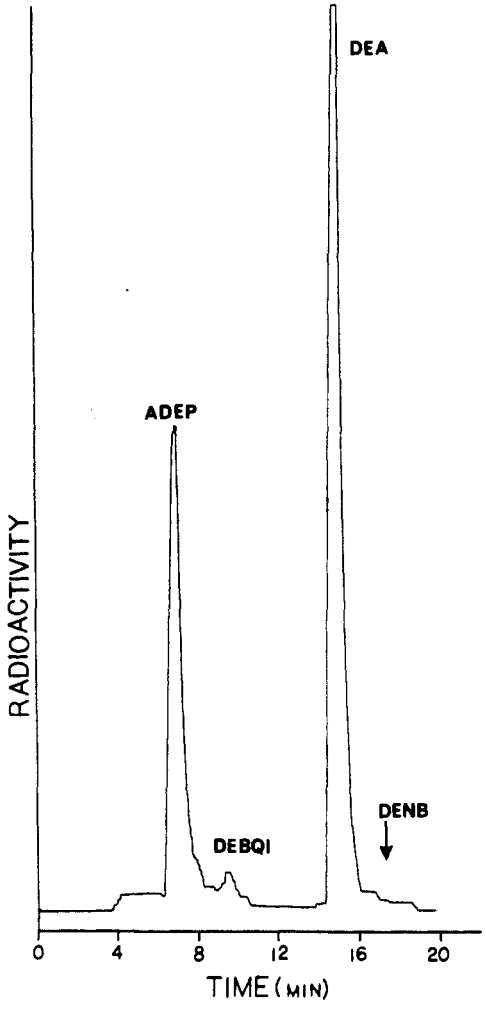

Figure 2. HPLC analysis with radioactivity detection of the products of DEA oxidation by rat liver microsomes. The arrow points to the expected retention time of DENB.

analysis confirmed that the ions at m/z 150 and 135 had resulted from losses of CH_3 and C_2H_6, respectively. In comparison, the EI spectrum of DEBQI (Figure 4) contained ions at m/z 163 ($M^{\cdot+}$, 55%), 165 ($M^{\cdot+}$ + 2, 10%), 148 ($M^{\cdot+}$ - 15, 15%), 135 ($M^{\cdot+}$ - 28, 18%), and 120 ($M^{\cdot+}$ - 15 - 28, 100%). Scanning high-resolution analysis confirmed that the ions at m/z 148 and 135 had resulted from the losses of CH_3 and CO, respectively. The facile loss of CO during EI mass spectral analysis is characteristic of benzoquinone derivatives (Budzikiewicz et al., 1967). Identical mass spectra were obtained from the analysis of authentic ADEP and DEBQI.

HPLC/MS analysis of the incubation extract employed filament ionization and acetonitrile–water as the solvents. The HPLC mass spectra for DEA, ADEP, and DEBQI each contained protonated molecular ions at 100% relative intensity and acetonitrile adduct ions (MH^+ + 41) at about 20% intensity. The MH^+ + 2 ions (m/z 166, 18%) were also observed in the spectrum of DEBQI; however, fragment ions were absent in all the spectra. No additional metabolites were detected in these analyses.

An aqueous methanol solution of synthetic ADEP was shown to easily autooxidize to DEBQI, which then slowly hydrolyzed to produce 2,6-diethylbenzoquinone (DEBQ). Since the amount of DEBQI that is produced from the enzymatic oxidation of DEA was quite variable from incubation to incubation, we propose that DEBQI arose at least partly from autooxidation rather than enzymatic oxidation. When an incubation mixture was deproteinated and centrifuged, and the supernatant was analyzed immediately by HPLC, the ratio of ADEP to DEBQI was 9 to 1, based on radioactivity quantitation. However, when the same incubation mixture was subjected to rigorous extraction with hexane, the same ratio decreased to 5 to 1. These results suggested that ADEP can be oxidized to DEBQI during the workup, presumably by molecular oxygen. The formation of DEBQ as a product of hydrolysis from DEBQI in these experiments was sometimes apparent during HPLC analysis but could not be confirmed by mass spectral analysis due to its low concentrations.

Although the oxidation of acetaminophen to *N*-acetyl-*p*-benzoquinone imine (NAPQI) has been proposed to be cytochrome P-450 catalyzed (Hinson et al., 1981), we believe that, in the case of ADEP, its oxidation to DEBQI was at least partly due to nonenzymatic reactions. As an electrophile, NAPQI reacts with glutathione (GSH) to produce 3-(glutathion-*S*-yl)acetaminophen, and the reactivity of NAPQI with thiols has been proposed as a mechanism of acetaminophen toxicity (Moore et al., 1985). DEBQI also underwent adduct formation in the presence of GSH. When DEA was incubated with liver microsomal enzymes fortified with both NADPH and GSH, a very polar metabolite was produced in addition to ADEP and DEBQI. Following HPLC purification, negative-ion FAB analysis of this polar metabolite produced ions at m/z 470 (50% relative intensity), which corresponded to the deprotonated molecular ions of 2-(glutathion-*S*-yl)-4-amino-3,5-diethylphenol (**10**) (Figure 5). The reduction of DEBQI back to ADEP was also observed in the presence of GSH.

Kimmel et al. (1986) reported the in vivo and in vitro metabolic conversion of alachlor to DEA and then to 2,6-diethylnitrosobenzene (DENB); the yield of DENB from the in vitro oxidation of DEA was reported to be 1–2%. Repeated attempts using Kimmel's procedures have failed to generate DENB in our in vitro incubations with [^{14}C]DEA; HPLC analysis with radioactivity detection did not produce a discernible peak at the retention time of authentic DENB (Figure 2). During the course of our experiments, we determined that DENB was extremely thermolabile and decomposed during capillary GC/MS analysis to six or more products (Wratten et al., 1987). The relative amounts of these degradation products were highly variable, and depending on the analytical conditions, 20% to >95% of DENB was estimated to be degraded. When extremely dilute solutions (0.1 ng/μL) of DENB were analyzed, as specified by Kimmel et al., the decomposition during GC/MS analysis was reduced. We believe that a better method of analysis for DENB is HPLC/MS. Synthetic DENB is stable during HPLC/MS analysis and produced protonated molecular ions (m/z 164) at 100% relative intensity. HPLC/MS analysis of the DEA enzymatic oxidation mixtures verified the presence of ADEP, DEBQI, and DEA; however, at the expected retention time of DENB, protonated molecular ions corresponding to DENB were not detected. With authentic DENB, the minimum detectable amount was about 30 ng/injection by HPLC/MS. Based upon the amounts of the incubation mixtures that were analyzed (50 μg equiv of DEA), the HPLC/MS analysis could have detected a 0.06% conversion of DEA to DENB.

Since DENB was extremely unstable during GC analysis, HPLC was used to determine the maximum amount of DENB that could have been produced from the oxidation of DEA by the liver microsomes. [^{14}C]DEA (6.4 μg, 200 000 dpm/μg) was incubated with liver microsomes as described above. At 0.25, 0.5, 1.0, and 2.0 h, aliquots (100

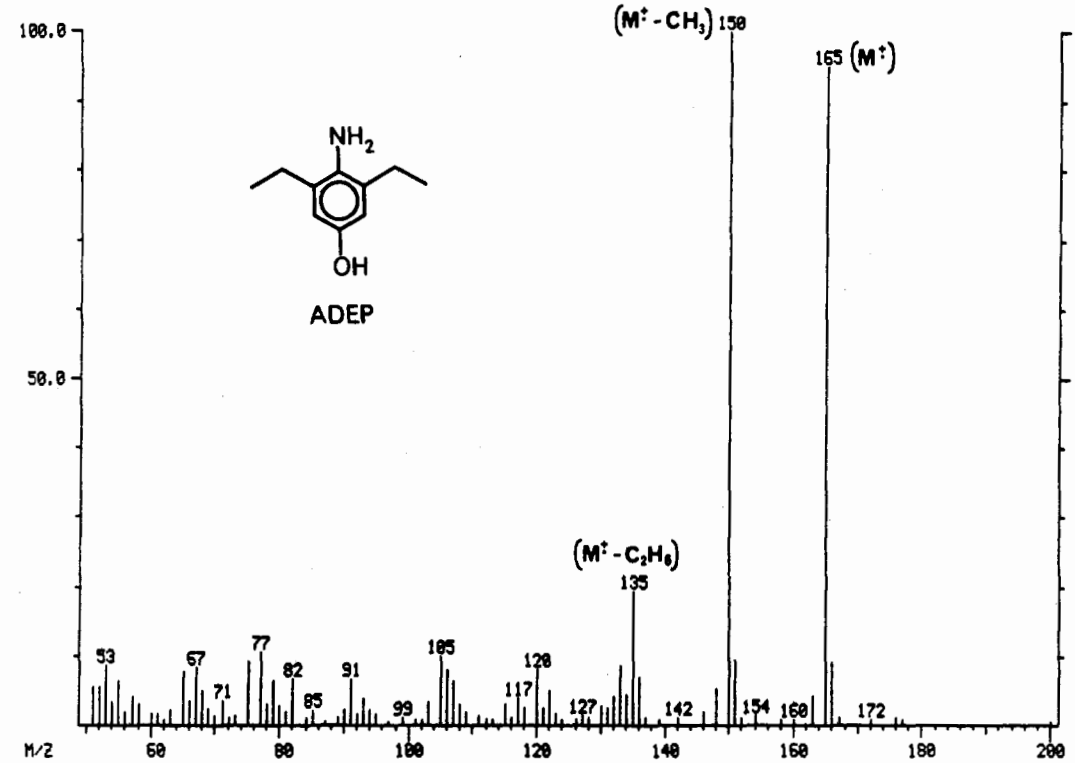

Figure 3. Electron impact mass spectrum of ADEP analyzed by GC/MS.

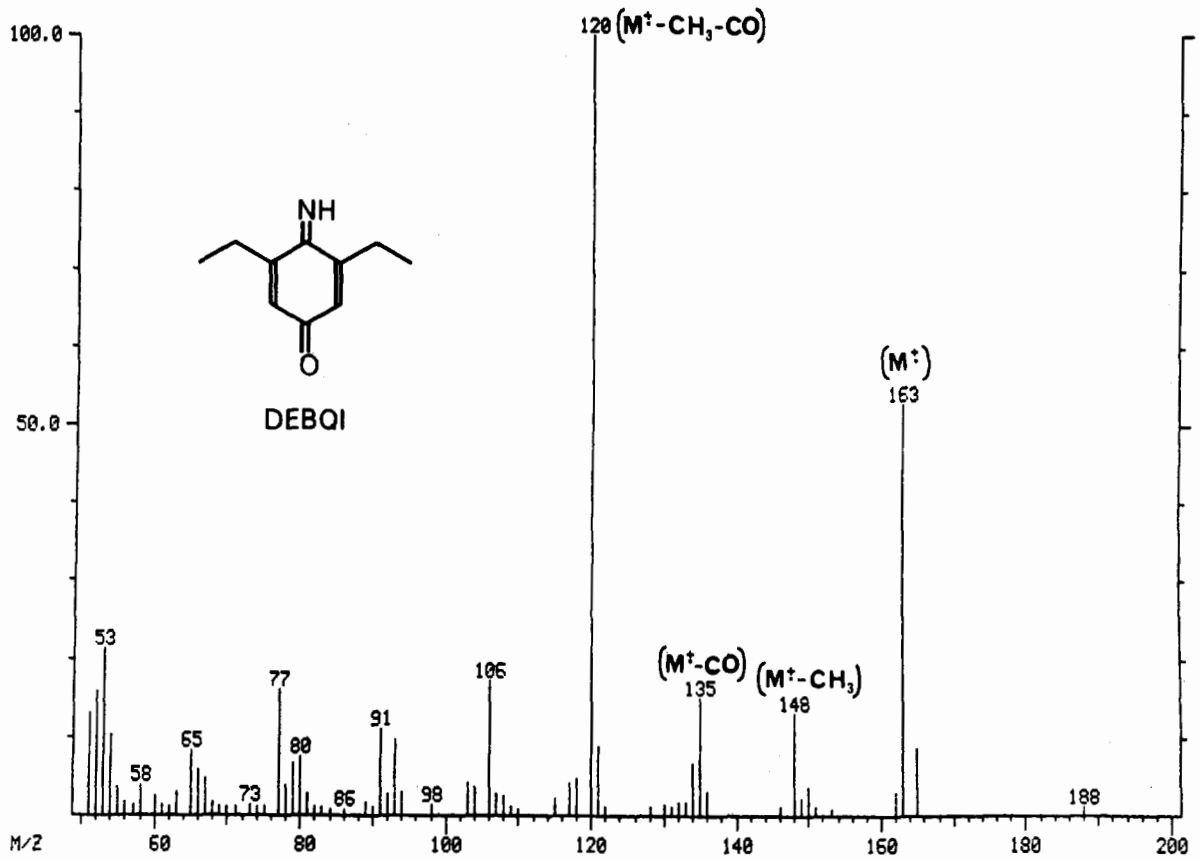

Figure 4. Electron impact mass spectrum of DEBQI analyzed by GC/MS.

μL) were removed and quenched with methanol. To aid in the recovery of any [^{14}C]DENB that might have been produced, a large excess of unlabeled authentic DENB (50 μg) was added to each of the aliquots, which were then centrifuged, and the supernatant was analyzed by HPLC. The HPLC effluent containing the authentic DENB was then collected and analyzed by liquid scintillation counting, which showed that these collections accounted for 0.27%, 0.21%, 0.17%, and 0.17% of the injected radioactivity for the four samplings ranging from 0.25 to 2 h. These levels of radioactivity were virtually indistinguishable from the background (0.17%), which was determined from an aliquot prior to the addition of NADPH in the same incubation and were 10-fold less than the yield of DENB re-

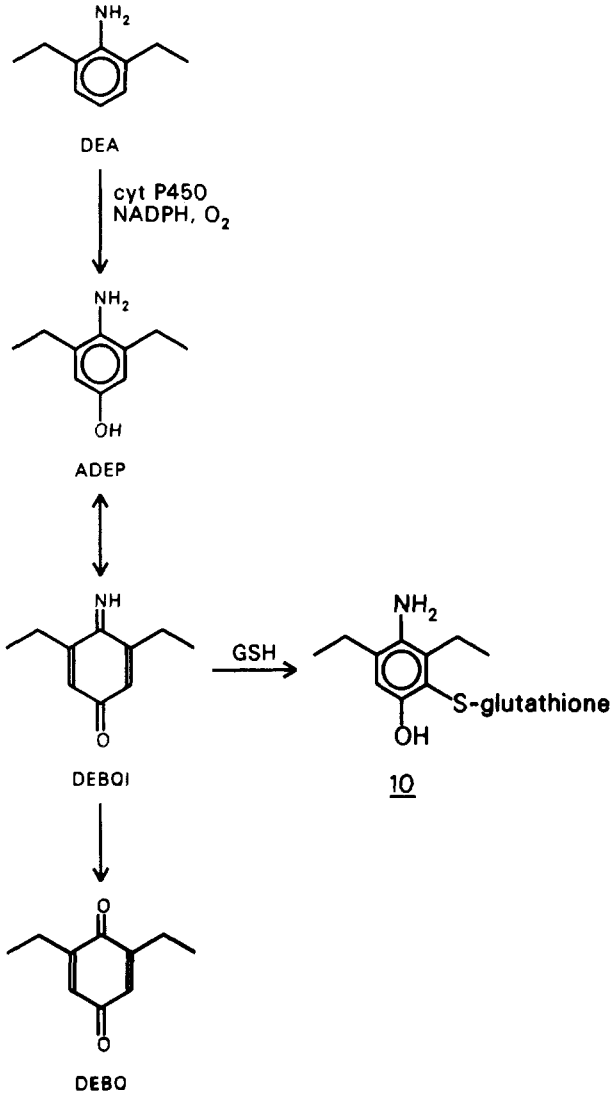

Figure 5. Metabolism of DEA by rat liver microsomal enzymes.

ported by Kimmel et al. (1986). In comparison, the level of ADEP that was formed in the same incubation accounted for 44%, 56%, 59%, and 63% of the injected radioactivity for the same time periods.

Figure 5 summarizes the metabolism of DEA by rat liver microsomal enzymes. Our results showed that, of the three potential sites of oxidation (N-oxidation, alkylhydroxylation, arylhydroxylation), DEA was exclusively metabolized by the rat liver microsomal enzymes to ADEP. This reaction was most likely catalyzed by the cytochrome P-450 monooxygenases. ADEP was then oxidized nonenzymatically to form DEBQI, and in the presence of GSH, DEBQI was conjugated with GSH or reduced to ADEP. Further hydrolysis of DEBQI produced DEBQ. Repeated attempts to detect the formation of DENB in the incubation by GC/MS, HPLC/MS, and HPLC with radioactivity detection were unsuccessful. Coincidentally, the molecular weight of the DEBQI (7; Figure 6) is identical with that of DENB (9); furthermore, Wratten et al. (1987) identified two other isomeric molecules, 11 and 12 (Figure 6), among the decomposition products of synthetic DENB formed during GC/MS analysis. Kimmel et al. (1986) assigned a GC/MS peak eluting after DEA as DENB; our studies showed that intact DENB eluted from GC/MS before DEA while isomeric DEBQI, 11, and 12 all eluted after DEA. It is conceivable that because of their identical molecular weights DEBQI, 11, or 12 could have been

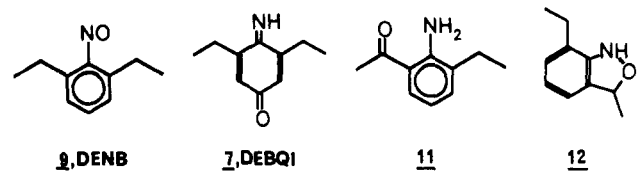

Figure 6. Isomers of DENB produced from its decomposition (11 and 12) and DEA microsomal oxidation (DEBQI).

mistakenly assigned as DENB during GC/MS/SIM analysis.

The N-oxidation of arylamines in the liver is mainly accomplished by the flavin mixed-fuction oxidases and the cytochrome P-450 monooxygenases (Cho et al., 1984). The activities of these two enzyme systems have been shown to be species, tissue, and substrate dependent. For example, aniline was not N-oxidized by the purified flavin-oxidase from porcine liver (Ziegler et al., 1973); however, it appeared to be N-oxidized by both the flavin and cytochrome P-450 oxidases of the rabbit liver (Heinze et al., 1970). The enzymatic N-oxidation of ortho-substituted alkylanilines such as DEA has not been extensively studied. Our in vitro studies using rat liver microsomal enzymes demonstrated that DEA was exclusively oxidized at the *p*-phenyl position to produce ADEP, and if DENB was produced, it could not be detected under our assay conditions. Taking into consideration the instability of DENB in solution (Wratten et al., 1987), its reported mutagenicity (Kimmel et al., 1986) requires further examination. Also of significance is a recent report by Pullin et al. (1985) that demonstrated that DEA was not carcinogenic during a 2-year feeding study in rats.

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