In Vitro Dehalogenation of *para*-Substituted Aromatic Halides in Rat Liver Preparations

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Abstract □ The *in vitro* dehalogenation of a series of *para*-substituted halobenzenes was studied using HPLC separation followed by scintillation counting or neutron-activation analysis. Microsomal and cytosolic deiodination were established for iodobenzene substrates whose *para*-substituents were CO_2H, CHO, NO_2, OH, and C_6H_5 but not for *para*-iodobenzonitrile. A nonglutathione cytosolic deiodinase was only indicated with 4-iodobiphenyl as the substrate. *In vitro* dehalogenation could not be established for 4-bromobiphenyl using neutron-activation analysis.

Keyphrases □ Dehalogenation—rat liver *in vitro* studies, microsomal, cytosolic □ Aromatic halides—effect of *para*-substitution on *in vitro* dehalogenation □ Glutathione—role in *in vitro* dehalogenation, *N*-ethylmaleimide inhibition

There has been considerable human exposure to aromatic halides due to their use in medicine as well as their widespread applications in industry and agriculture. In addition to the medicinal use of thyroxine and iodohydroxyquin preparations, acyl iodides have been employed as radiopaque diagnostic agents. Also, ^{131}I- or increasingly, ^{123}I-labeled aromatic iodides have been synthesized as potential tumor-imaging agents (1–4). Polychlorinated and polybrominated biphenyls are ubiquitous environmental pollutants with well-established toxicities (5–10).

The possibility of contact with these compounds is evident and has prompted numerous investigations, especially with the biphenyl compounds. *In vivo* dehalogenation plays a small but significant role in the metabolism of these compounds (11–13). Therefore, it was basic to an understanding of their potential toxicity to study the mechanism and extent of *in vitro* dehalogenation in the rat by direct measurement of liberated halide. *para*-Substituted aromatic halides labeled with iodine-125 or chlorine-36, or containing unlabeled bromine or chlorine, were chosen as the model compounds.

EXPERIMENTAL SECTION

Apparatus—The liquid chromatograph consisted of a microprocessor controller[1], two pumps[2], a dynamic-flow mixer[3], and an injection valve[4]. A reverse-phase C_{18} column[5] was used for radiohalide detection by either a radiochemical detector in a liquid scintillation mode[6] or by liquid scintillation counting[7] of the eluant. A cumene-based cocktail[8], at a flow rate of 3.0 mL/min, was used with the radiochemical detector, while a xylene-based cocktail[9] was used for liquid scintillation counting of the eluant.

Synthesis—^{125}I-labeled compounds, except for [4,4′-^{125}I]diiodobiphenyl, were synthesized by an exchange reaction using a melt procedure (14). Labeled 4,4′-diiodobiphenyl was synthesized as previously described (15). Radiochemical purity was established by reverse isotopic dilution analysis and HPLC.

General Melt Procedure—The unlabeled iodo compound (25 mg) and acetamide (100 mg) were added to a flask followed by sodium[^{125}I]iodide[10] (1–2 mCi) in acetone. Sodium thiosulfate (10^{-7} M, 20 μL) and ammonium chloride (10^{-7} M, 20 μL) were also added to prevent oxidation of the iodide and to prevent degradation of the reactant, respectively. Reaction conditions for specific compounds and their yields are outlined in Table I. Water (4 mL) was added, and the mixture was extracted with chloroform (4 × 4 mL), dried over sodium sulfate, and evaporated to dryness under a stream of nitrogen. The solid was dissolved in a small volume (~1 mL) of chloroform and passed through a small silica gel column[11] to remove inorganic iodide-125. The eluant was evaporated using nitrogen, and the solid was recrystallized in methanol (except for the iodobiphenyl compounds, which were recrystallized in ethanol).

Reverse Isotopic Dilution Analysis—A known amount of radiolabeled material was added to 500–1000 mg of accurately weighed unlabeled material. Mixtures were recrystallized until a constant specific activity of ±5% was achieved. From each recrystallization, three 10-mg aliquots, accurately weighed, were counted (Table I).

Radiochemical Purity by Liquid Chromatography—A reverse-phase C_{18} column[12], with methanol–water (90:10) as the mobile phase, was used to determine the purity of the ^{125}I-labeled substrates. The presence of any inorganic iodide-125 in the ethanolic or buffer solutions is listed in Table I.

Pretreatment of Animals—Male Sprague–Dawley rats[13] (250–300 g) were quarantined for 2 d before a single intraperitoneal injection of a commercial mixture of polychlorinated biphenyls[14] [500 mg/kg (16)] in corn oil. Rats had free access to food and water for 4 d, after which time the food was removed. The rats were killed on day 5 by asphyxiation using carbon dioxide.

Liver Fractions—Preparation of microsomal and cytosolic fractions were carried out as previously described (15). The procedure of Levine and Murphy (17) was used for the preparation of glutathione-depleted cytosol.

Incubation Procedures—The incubation conditions were modifications of those previously described (15), and studies with *N*-ethylmaleimide[15] and glutathione-depleted cytosol were performed. Mixtures of 20 μL of the ethanolic substrate solution (containing at least 40,000 cpm of substrate), 460 μL of homogenate, and 20 μL of buffer (0.01 M Na_2HPO_4–0.25 M sucrose, pH 7.4) or microsomal additive solution were incubated for 2 h at 37°C. Microsomal additive solutions consisted of magnesium chloride (15 μmol), nicotinamide (20 μmol), and NADPH (0.05 μmol) contained in 20 μL of buffer. *N*-Ethylmaleimide was added to selected substrate solutions to inactivate cytosolic glutathione such that the final concentration of imide was 0.0025 M. Five experimental and three control values were run for each experiment. Controls consisted of immersing the homogenate for 5 min in boiling water, cooling, and then following the incubation procedure as above.

Analytical Procedures—*Deiodination Studies*—The enzymatic reaction was stopped by the addition of 150 μL of 20% trichloroacetic acid. Following centrifugation (2000×*g*) and removal of the supernatant, the pellet was washed with 0.5 mL of buffer. After recentrifugation the two supernatants were combined, and the total supernatant was centrifuged and filtered through a 0.45-μm membrane[16] prior to injection into the liquid chromatograph. Samples were stored frozen until HPLC analysis.

To separate inorganic iodide from unreacted substrates and metabolites, 1.0 mL of *in vitro* solution was injected into the liquid chromatograph and eluted with a mobile phase of methanol–water (20:80) using a reverse-phase C_{18} column[5]. With the radiochemical detector, cpm measurements were taken every minute for 5 min after injection. When a scintillation counter was used, the first 4 mL of eluant was collected in a scintillation vial and counted for 5 min following the addition of 1 mL of methanol. The methanol was used to preserve a homogeneous solution with the cocktail (14 mL). Between injections, the chromatograph was flushed with methanol (30 mL) and equilibrated again with the solvent system.

[1] Model 421; Altex, Berkeley, Calif.
[2] Model 110A; Altex, Berkeley, Calif.
[3] Altex, Berkeley, Calif.
[4] Model 7125, 2.0-mL loop; Rheodyne, Berkeley, Calif.
[5] RP-18, 30 × 4.6 mm i.d., Brownlee, Santa Clara, Calif.
[6] Model Flo-One DR; Radiomatic, Tampa, Fla.
[7] Model 9000; Beckman, Berkeley, Calif.
[8] Flo-Scint II; Radiomatic, Tampa, Fla.
[9] ACS, Amersham, Arlington Heights, Ill.
[10] New England Nuclear, Boston, Mass.
[11] Sep-Pak; Waters Associates, Milford, Mass.
[12] ODS-3, 250 × 4.6 mm i.d.; Whatman, Clifton, N.J.
[13] Charles River, Portage, Mich.
[14] Aroclor 1254; Monsanto, St. Louis, Mo.
[15] Sigma, St. Louis, Mo.
[16] Metricel GA-6; Gelman Scientific, Ann Arbor, Mich.

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Table I—Synthesis of ^{125}I-Labeled Substrates [a]

Compound	Reaction Conditions: Max. Temp., °C (for Time, h)	Reaction Conditions: Total Heating Time, h	Specific Activity, mCi/mmol [b]	Radiochemical [c] Purity, %	Inorganic Iodine-125, %: Ethanol	Inorganic Iodine-125, %: Buffer [d]
p-Iodobenzoic Acid	180–190 (1)	2.5	4.13 (12.5)	95.4 ± 5.2	0.0152	0.0139 ± 0.00652 [e]
p-Iodobenzaldehyde	110–120 (1)	2.5	0.111 (3.52)	95.3 ± 2.4	0.668 ± 0.0848	0.903 ± 0.070
p-Iodobenzonitrile	110–120 (1.5)	3.0	0.427 (1.75)	99.5 ± 3.0	0.505 ± 0.00402	0.577 ± 0.0724
p-Iodonitrobenzene	145 (1.5)	3.5	0.973 (3.0)	91.6 ± 1.5	0.0949 ± 0.0403	0.0747 ± 0.0561
p-Iodophenol	100–110 (1.25)	3.5	7.022 (17.47)	98.1 ± 0.75	0.151 ± 0.0244	0.333 ± 0.146
4-Iodobiphenyl	130–135 (1.5)	3.0	1.734 (13.86)	98.4 ± 1.2	0.0708 ± 0.00465	0.108 ± 0.0075
4,4′-Diiodobiphenyl [f]	225 (48)	48.5	17.97 (82.5)	98.6 ± 1.6		

[a] Synthesized by the method of Elias and Lotterhos (14). [b] Radiochemical yield, expressed as percentage, in parentheses. [c] Reverse isotopic dilution. [d] Incubation in pH 7.4 phosphate buffer for 2 h at 37°C. [e] Mean of three experimental values ± SD. [f] Synthesized as previously described (16).

Debromination and Dechlorination Studies of Unlabeled Substrates—The enzymatic reaction was stopped with the addition of 150 μL of 35% H_2SO_4. The aforementioned procedure for iodide was then followed except that Tris-sucrose buffer was used; the HPLC procedure for separation of inorganic bromide and chloride was identical to that for iodide. The eluant (4 mL) was condensed by lyophilization after addition of 160 μL of concentrated ammonium hydroxide to prevent charring.

For bromide analysis, the residue was transferred to quartz tubes[17] with 1.0 mL of deionized water. Following lyophilization overnight, ≤0.5 mL of deionized water was added and the tubes were sealed. They were placed in the reactor at a neutron flux of 1.5×10^{13} neutrons/cm^2/s for 50 h. Bromide was determined by gamma-ray analysis of bromide-82 ($t_{1/2}$ = 35.34 h, 554.3, 619.0, and 776.5 keV) using two Canberra 22% coaxial Ge (Li) detectors and two multichannel analyzers[18].

For chloride analysis, the lyophilized material was transferred to small polyethylene tubes[19] with deionized water (0.25 mL). Tubes were placed in the reactor core at a flux of 1.5×10^{13} neutrons/cm^2/s for 15 min. Chloride was determined as chloride-38 ($t_{1/2}$ = 37.3 min, 1642.0 and 2167.5 keV) with the aforementioned instrumentation.

Glutathione-*S*-Transferase Assay—Glutathione-*S*-transferase was assayed by the method of Habig *et al.* (18). The concentration of *p*-nitrobenzyl chloride in the final solution was 0.00025 M, and the concentration of glutathione, when required, was 0.005 M in 0.5 M Na_2HPO_4 buffer, pH 7.4. The substrate was delivered in 25 μL of acetonitrile. Cytosol (100 μL) was used and UV absorbance measurements were taken at 310 nm every minute for 10 min.

Glutathione Determination—Glutathione was determined by the Ellman thiol assay (19) as modified by Benke *et al.* (20). A glutathione stock solution (0.002 M) was prepared in 0.1 M Na_2HPO_4 buffer (pH 8.0) containing 0.5% trichloroacetic acid and 0.001 M EDTA (tetrasodium salt). Four hundred microliters of stock solution was added to 4.55 mL of buffer, followed by the addition of 50 μL of 0.01 M 5,5′-dithiobis(2-nitrobenzoic acid). The absorbance was measured at 420 nm

RESULTS AND DISCUSSION

Analytical System—A reverse-phase HPLC technique similar to the systems previously reported for iodide (21, 22) was developed for separating inorganic halide from unreacted substrates and metabolites. Liquid scintillation counting of the eluant, following injection of sodium [^{36}Cl]chloride[10] in a simulated incubation mixture, resulted in quantitative recovery of inorganic chloride-36. Recovery of chloride-36 was independent of solvent composition from 10 to 90% methanol.

The analysis of inorganic iodide-125 required that carrier sodium iodide be added to the samples. Quantitative recovery was independent of solvent composition from 10 to 90% methanol. *para*-Iodobenzoic acid was chosen to determine the optimal solvent composition because resolution of this polar substrate and inorganic iodide could pose a difficult problem. Methanol–water (20:80) was suitable for all the substrates since complete resolution of inorganic and organically bound iodide was obtained in a minimal amount of time (~5 min).

Stability of Substrates—*In vitro* dehalogenation was expected to liberate trace quantities of halide; therefore, the purity of the labeled substrates, and the stability of the carbon–iodine-125 bond in ethanolic substrate solution and in buffer under incubation conditions, was of crucial importance if statistically significant levels of deiodination were to be obtained. Table I shows the percent inorganic iodide-125 found for the substrates in ethanol and in buffer under incubation conditions. The percentage of inorganic iodide in ethanol was determined by injection of an aliquot of an ethanolic solution of substrate containing 500 μg of carrier sodium iodide.

All solutions were freshly prepared and injected into the chromatograph within 5 min of preparation. Deiodination in buffer was carried out by incubating an aliquot of ethanolic substrate solutions in pH 7.4 sodium phosphate buffer (0.010 M) under the same conditions used in the *in vitro* experiments. HPLC conditions were also as described for *in vitro* dehalogenation studies.

Deiodination in ethanol was about the same as that in buffer under incubation conditions. Deiodination of [*p*-^{125}I]iodobenzaldehyde was statistically greater (Student's *t* test, $p \leq 0.05$) in buffer than in ethanol; there was no statistical difference in deiodination of the phenol in ethanol or buffer.

Dechlorination and Debromination Studies Using Neutron-Activation Analysis—Neutron-activation analysis was an attractive alternative to using radiolabeled substrates for *in vitro* debromination and dechlorination studies. Therefore, a feasibility study was undertaken using simulated incubation mixtures containing ammonium bromide or ammonium chloride and the reverse-phase HPLC conditions described above. These samples contained bromide and chloride equivalent to 0.5, 1.0, and 2.0% biotransformations, based on the incubation of 100 μg of 4-bromobiphenyl or 4-chlorobiphenyl. Bromide analysis at 554.0 and 776.9 keV was used. Results of analysis at 619.0 keV were not used due to interference from another radioisotope. HPLC analysis at either 554.0 or 776.9 keV of blanks containing no ammonium bromide did not show statistically significant bromide levels; therefore, bromide contamination as a result of the workup and analysis was not a problem. Statistically significant levels of bromide were observed for all three of the simulated biotransformations.

Neutron-activation analyses at 1641.3 and 2165.9 keV of simulated, dechlorinated samples indicated high background levels which were consistent with known interference in this method by other elements, particularly sodium and potassium in the measurement of chloride. It was estimated that there would have to be at least a 3% biotransformation for detection of chloride in this background. Therefore, while neutron-activation analysis of bromide was a viable technique for study of debromination, this technique was not useful under the conditions of our incubations for chloride measurements.

Neutron-activation analysis of the HPLC effluent following injection of the supernatant of the incubation medium of 4-bromobiphenyl with cytosol (for three sets of determinations) gave 0.206 ± 0.032% *versus* 0.169 ± 0.032% for the respective control at 553.8 keV and 0.209 ± 0.032% *versus* 0.156 ± 0.019% at 776.0 keV. Neither measurement, compared to the respective control, was statistically significant at $p \leq 0.05$.

Debromination (~1%) of 4,4′-dibromobiphenyl has been reported in the rabbit (12) and pig (13). However, Safe *et al.* (12) did not detect any debrominated metabolites of 4-bromobiphenyl after administration to pigs or rabbits. Analysis of the *in vitro* incubation of tritiated 4-bromobiphenyl with rabbit liver microsomes also showed no debrominated metabolite (12). Thus, while debromination is known to occur, it has not been detected with 4-bromobiphenyl previously or in this study. Analysis of *in vitro* debromination studies was hampered by low substrate solubility. *In vitro* debromination was expected to be less than deiodination based on aromatic carbon–bromine bond strength compared with that of the carbon–iodine bond.

Microsomal Deiodination—The results of deiodination in microsomes are shown in Table II. Each substrate exhibited statistically significant deiodination above its control value except for [*p*-^{125}I]iodobenzonitrile. The relatively high variance of the control values and the low amount of substrate activity prevented detection of a statistically significant difference in deiodination between microsomal samples and the respective controls. Control values, in general, agreed with those found in Table I for incubation in buffer.

Deiodination of the substrates was ≥ ~2%, and there was no unique specificity of the microsomes for any of the substrates. The results for [*p*-^{125}I]-iodonitrobenzene and [4-^{125}I]iodobiphenyl generally agreed with previous

[17] T21 Supersil-7 mm; Harris-Amersil Co., Sayerville, N.J.
[18] Models-4420 and 6660; Nuclear Data, Schaumberg, Ill.
[19] Cole-Palmer, Chicago, Ill.

Table II—Percent Deiodination of p-^{125}I—C_6H_4—R

R	Microsomal		Cytosolic					
	Experimental	Control	No additives	Control	*N*-ethyl-maleimide	Control	Glutathione-Depleted[a]	Control
—CO_2H	0.225 ± 0.0162 (4)[b,c]	0.0332 ± 0.0120 (3)	0.244 ± 0.0079 (5)[b]	0.0046 ± 0.0081 (3)	0.175 ± 0.0994 (4)[d]	0.172 ± 0.00078 (2)[d]	—[e]	—
—CHO	1.37 ± 0.0655 (4)[b]	0.848 ± 0.0685	1.48 ± 0.126 (5)[b]	1.08 ± 0.102 (3)	0.854 ± 0.134 (5)	0.951 ± 0.151 (3)	—	—
—CN	0.625 ± 0.0850 (5)	0.594 ± 0.0680	0.695 ± 0.090 (5)[b]	0.550 ± 0.051 (3)	0.451 ± 0.079 (5)	0.576 ± 0.064 (3)	—	—
—NO_2	1.97 ± 0.499 (4)[b]	0.181 ± 0.0393	28.8 ± 3.02 (5)[b]	1.51 ± 0.0839 (3)	0.498 ± 0.0645 (5)	0.487 ± 0.046 (3)	—	—
—OH	1.32 ± 0.345 (4)[b]	0.459 ± 0.0540	1.89 ± 0.194 (4)[b]	0.857 ± 0.109 (3)	2.21 ± 0.068 (5)[b]	0.735 ± 0.105 (3)	0.358 ± 0.029 (5)	0.381 ± 0.020 (3)
—C_6H_5	1.12 ± 0.348 (5)[b]	0.143 ± 0.0108	0.273 ± 0.0087 (5)[b]	0.182 ± 0.0241 (3)	0.213 ± 0.0087 (5)[b]	0.170 ± 0.0184 (3)	0.137 ± 0.006 (5)[b]	0.113 ± 0.011 (3)
—$C_6H_4{}^{125}I$[f]	0.00	0.00	—	—	—	—	—	—

[a] Depleted by the method of Levine and Murphy (17). [b] Statistically significant above control values ($p \leq 0.05$). [c] Percent deiodination ±*SD*, with the number of determinations in parentheses. [d] Adjusted enzyme activity from a different source for this cytosolic fraction, by comparison of cytosolic activities with the nitro compound as the substrate. [e] Not determined. [f] Substrate could not be incorporated into the medium without excessive loss of enzymatic activity.

results determined in this laboratory: 2.5 ± 0.52% for labeled *p*-iodonitrobenzene and 0.6 ± 0.04% for [4-^{125}I]iodobiphenyl (15).

The microsomal deiodination of these compounds can be explained by considering them as substrates for the mixed-function oxygenase system with arene oxide formation at the iodocarbon. The possible loss of iodide during a subsequent "N.I.H. shift" (23) involving this arene oxide would be analogous to the mechanism proposed for the loss of bromide or chloride in polyhalogenated biphenyl compounds (12, 13).

No correlation was found between microsomal deiodination and the electron-withdrawing character of *para*-substituents, as indicated by their Hammett values (24). Furthermore, no correlation was found between microsomal deiodination and the lability of the carbon-iodine bond as evidenced by the radiochemical yield in the exchange reaction used in their preparation.

Cytosolic Deiodination—Results of cytosolic deiodination are also shown in Table II. Six of the seven substrates exhibited deiodinations that were statistically significantly greater than the control values ([4,4′-^{125}I]diiodobiphenyl did not). [*p*-^{125}I]Iodonitrobenzene deiodinated to the greatest extent above the respective control value, indicating a unique specificity of glutathione-*S*-transferase for the nitro compound. The extent of this transformation, although large, was not unexpected. The metabolism of *p*-chloronitrobenzene resulted in ~7% of the dose being excreted as the mercapturic acid (25). The more labile carbon-iodine bond would facilitate increased deiodination. Cytosolic control values generally agreed with those in buffer and ethanol (Table I), although control values for the benzoic acid, nitro, 4-iodobiphenyl, and phenol substrates were significantly higher than their deiodination in buffer. Nonenzymatic glutathione conjugation of the nitro compound was believed to account for the high amount of deiodination found in the respective control. The reduction in deiodination when free glutathione levels were reduced with the addition of *N*-ethylmaleimide would further substantiate this belief. Pabst *et al.* (26) have established that nonenzymatic conjugation of glutathione with *p*-nitrobenzyl chloride and 1,2-dichloro-4-nitrobenzene was significant at or near physiological pH in the rat. Deiodination of the *p*-nitro and 4-iodobiphenyl substrates agreed well with values previously reported from this laboratory (15). No correlation was found between cytosolic deiodination and the electron-withdrawing character of the *para*-substituent for the acid, aldehyde, nitrile, nitro, phenol, or 4-iodobiphenyl substrates as measured by their Hammett values (24). There was also no correlation with the radiochemical yield of the syntheses of these compounds.

Kraus (27) reported the dechlorination of α-hexachlorocyclohexane in rat liver cytosol by a mechanism that was not dependent on glutathione and by a glutathione-*S*-transferase mechanism. Our previous studies also indicated a second cytosolic dehalogenase (16). An efficient means of inhibiting glutathione-*S*-transferase was sought in order to explore the nature of this second cytosolic dehalogenase system.

N-Ethylmaleimide is known to bind to thiols (28). [p-^{125}I]Iodonitrobenzene was incubated as described with 0.1–40.0 mM *N*-ethylmaleimide to determine the optimal concentration for the inhibition of glutathione-*S*-transferase. Deiodination decreased as the concentration of *N*-ethylmaleimide increased up to 2.5 mM. Increasing its concentration >2.5 mM resulted in no statistically significant decrease in deiodination. Thus, 2.5 mM *N*-ethylmaleimide was chosen as the optimal concentration in the final incubation mixture. Confirmation of the inhibition of glutathione-*S*-transferase was also established by measuring the concentration of glutathione in cytosol with and without *N*-ethylmaleimide. The standard Ellman thiol assay (19) as modified by Benke (20) showed that glutathione was below detectable levels with the addition of 2.5 mM *N*-ethylmaleimide.

Results of cytosolic deiodination with *N*-ethylmaleimide present are shown in Table II. [4-^{125}I]Iodobiphenyl and [*p*-^{125}I]iodophenol were the only substrates to exhibit statistically significant deiodination above the respective control values.

These two possible indications of a second nonglutathione cytosolic deiodinase were also studied by the use of an anion-exchange preparation (17) of glutathione-depleted cytosol. Glutathione concentration was again assayed by the Ellman procedure (20) and found to be below detectable limits. Further evidence of the efficiency of glutathione removal was demonstrated by determining the glutathione-*S*-transferase activity of intact and glutathione-depleted cytosol. The procedure of Habig *et al.* (18) with *p*-nitrobenzyl chloride as the substrate was used to follow the UV absorbance of the glutathione conjugate. Formation of the conjugate did not increase with time when glutathione-depleted cytosol was used. When the depleted cytosol was reconstituted with glutathione (5 mM), the rate of formation of the conjugate was not statistically different from that of intact cytosol. Therefore, this depletion procedure inhibited glutathione activity, but did not adversely affect glutathione-*S*-transferase.

The results of deiodination of [*p*-^{125}I]iodophenol and [4-^{125}I]iodobiphenyl using glutathione-depleted cytosol are shown in Table II. The deiodination of the biphenyl was statistically significant above the respective control value, but this was not the case for the phenolic compound. Thus, this confirmation of the presence of a cytosolic nonglutathione dehalogenase could only be established for the iodobiphenyl substrate. The microsomal and cytosolic glutathione systems were the more generally applicable deiodination mechanisms.

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Determination of Bupropion and Its Major Basic Metabolites in Plasma by Liquid Chromatography With Dual-Wavelength Ultraviolet Detection

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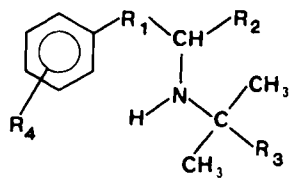
Abstract □ A method for the determination of bupropion and its three major basic metabolites in plasma is described. Following an extraction from alkaline plasma into 1.5% v/v isoamyl alcohol in *n*-heptane, a portion of the acid-backwashed extract was injected onto a column packed with trimethylsilyl reverse-phase material and eluted with a phosphate buffer–acetonitrile (80:20) mobile phase containing an ion-pairing reagent and triethylamine. The compounds were detected with a dual-wavelength UV detector (214 and 254 nm) to optimize sensitivity and facilitate simultaneous detection. The method provides an absolute recovery of ~85% for bupropion and ~98% for the metabolites. Day-to-day reproducibility did not exceed 4.0% for all compounds. The detection limits were ~5 ng/mL for bupropion and 100 ng/mL for the major metabolites. The limit of 100 ng/mL for metabolite quantitation is imposed by the internal standard concentration selected for steady-state studies. In single-dose pharmacokinetic studies, 10% of the steady-state concentration of internal standard was used; this permitted a 10-ng/mL lower limit of detection. Steady-state plasma levels of bupropion and the metabolites from eight different patients are presented.

Keyphrases □ Bupropion—metabolites, HPLC, dual-wavelength UV detection □ Antidepressants—bupropion and its metabolites, HPLC, dual-wavelength UV detection

Bupropion hydrochloride[1] [(±)-2-*tert*-butylamino-3′-chloropropiophenone hydrochloride], a chemically unique antidepressant currently undergoing clinical evaluation, appears to be free of any significant anticholinergic or cardiovascular effects (1–3). The assay of bupropion in biological fluids has been limited to RIA (4) and high-performance liquid chromatography (HPLC) (5). The RIA procedure appears to be sensitive (<1 ng/mL) and specific for bupropion, whereas the HPLC method is less sensitive (50 ng/mL). However, neither method quantitates metabolites.



I: R_1 = C=O, R_2 = R_3 = CH_3, R_4 = 3-Cl
II: R_1 = C=O, R_2 = CH_3, R_3 = CH_2OH, R_4 = 3-Cl
III: R_1 = CHOH, R_2 = R_3 = CH_3, R_4 = 3-Cl (*erythro*)
IV: R_1 = CHOH, R_2 = R_3 = CH_3, R_4 = 3-Cl (*threo*)
V: R_1 = CHOH, R_2 = CH_3, R_3 = CH_2OH, R_4 = 3-Cl (*erythro*)
VI: R_1 = C=O, R_2 = C_2H_5, R_3 = CH_3, R_4 = 4-F
VII: R_1 = C=O, R_2 = C_3H_7, R_3 = CH_3, R_4 = 4-F

Current interest in biologically active metabolites of psychotropic drugs led us to develop an HPLC procedure which separates and quantitates bupropion and its major basic metabolites in plasma with dual-wavelength UV detection.

EXPERIMENTAL SECTION

Apparatus—Chromatography was performed with a dual-piston solvent delivery pump[2] with either a manual injector[3] or an automatic sample processor[4]. The column was 25 cm × 4.6 mm i.d. packed with 5-μm particle size trimethylsilyl material[5]. The effluent was monitored at 254 and 214 nm by

[1] Wellbutrin.

[2] Model 6000A; Water Associates, Milford, Mass.
[3] U6K Injector; Waters Associates.
[4] Wisp 710B; Waters Associates.
[5] LC-1; Supelco, Bellafonte, Pa.

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